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(54) Title: ANTI A BETA ANTIBODY FORMULATION

(57) Abstract: The present invention provides formulations for maintaining the stability of A β binding polypeptides, for example, A β antibodies. Exemplary formulations include a tonicity agent such as mannitol and a buffering agent or amino acid such as histidine. Other exemplary formulations include an antioxidant in a sufficient amount as to inhibit by-product formation, for example, the formation of high molecular weight polypeptide aggregates, low molecular weight polypeptide degradation fragments, and mixtures thereof. The formulations of the invention optionally comprise a tonicity agent, such as mannitol, and a buffering agent or amino acid such as histidine. The formulations are suitable for several different routes of administration.

ANTI A BETA ANTIBODY FORMULATION

RELATED APPLICATIONS

This application claims the benefit of US provisional patent application bearing
5 Serial No. 60/ 648,631 (filed January 28, 2005), entitled "Anti A Beta Antibody
Formulation". The entire content of the above-referenced application is incorporated
herein by reference.

BACKGROUND OF THE INVENTION

10 Alzheimer's disease ("AD") is a neurodegenerative disorder characterized by the
occurrence of amyloid plaques, neurofibrillary tangles and significant neuronal loss. β -
Amyloid protein (also referred to as the A β peptide), the main component of senile
plaques, has been implicated in the pathogenesis of Alzheimer's disease (Selkoe (1989)
Cell 58:611-612; Hardy (1997) *Trends Neurosci.* 20:154-159). β -Amyloid has been
15 shown to be both directly toxic to cultured neurons (Lorenzo and Yankner (1996) *Ann.*
NY Acad. Sci. 777:89-95) and indirectly toxic through various mediators (Koh et al.
(1990) *Brain Research* 533:315-320; Mattson *et al.* (1992) *J. Neurosciences* 12:376-
389). Additionally, *in vivo* models, including the PDAPP mouse and a rat model have
linked β -amyloid to learning deficits, altered cognitive function, and inhibition of long-
20 term hippocampal potentiation (Chen et al. (2000) *Nature* 408:975-985; Walsh et al.
(2002) *Nature* 416:535-539). Therefore, a great deal of interest has focused on therapies
that alter the levels of β -amyloid to potentially reduce the severity or even abrogate the
disease itself.

One AD treatment strategy that has recently emerged in response to successful
25 studies in PDAPP mouse and rat experimental models, is that of immunization of
individuals to either provide immunoglobulins such as antibodies (as in the case of
passive immunization, wherein therapeutic immunoglobulins are administered to a
subject) or to generate immunoglobulins (active immunization, wherein the immune
system of a subject is activated to produce immunoglobulins to an administered antigen)
30 specific to β -amyloid. These antibodies would in turn help reduce the plaque burden by
preventing β -amyloid aggregation (Solomon *et al.* (1997) *Neurobiology* 94:4109-4112)
or stimulating microglial cells to phagocytose and remove plaques (Bard *et al.* (2000)

Nature Medicine 6: 916-919). Further by way of example, a humanized anti A β peptide IgG1 monoclonal antibody (a humanized 3D6 antibody) can effectively treat AD by selectively binding human A β peptide.

For a protein, and in particular, an antibody, to remain biologically active, a
5 formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (*i.e.*, any process which involves modification of the protein by bond
10 formation or cleavage resulting in a new chemical entity) or physical instability (*i.e.*, changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. For a general review of stability of protein pharmaceuticals, see, for example, Manning, *et al.* (1989) *Pharmaceutical Research* 6:903-918. In
15 addition, it is desirable to maintain stability when carrier polypeptides are not included in the formulation.

While the possible occurrence of protein instabilities is widely appreciated, it is impossible to predict particular instability issues for a particular protein. Any of these instabilities can potentially result in the formation of a polypeptide by-product or
20 derivative having lowered activity, increased toxicity, and/or increased immunogenicity. Indeed, polypeptide precipitation can lead to thrombosis, non-homogeneity of dosage form and immune reactions. Thus, the safety and efficacy of any pharmaceutical formulation of a polypeptide is directly related to its stability.

Accordingly, there continues to exist a need for formulations that not only
25 maintain the stability and biological activity of biological polypeptides, for example, A β binding polypeptides, upon storage and delivery, but are also suitable for various routes of therapeutic administration.

SUMMARY OF THE INVENTION

30 The present invention provides formulations designed to provide stability and to maintain the biological activity of an incorporated biologically active protein, in particular A β binding proteins or polypeptides, such as, for example, A β antibodies or

fragments or portions thereof. The invention further provides polypeptide formulations, such as, for example, stabilized liquid polypeptide formulations that are resistant to the formation of undesired polypeptide by-products.

The integrity of antigen-binding polypeptides for therapeutic use is especially
5 important because if the polypeptide forms by-products, for example, aggregates or degradation fragments during storage, bioactivity may be lost, thereby jeopardizing the therapeutic activity of the molecule per unit dose. In addition, there is an acute desire to stabilize therapeutic polypeptides intended for specialized functions, for delivery and use in certain biological indications, for example, treating neurodegenerative conditions,
10 where a polypeptide must traverse the blood-brain-barrier (BBB) and bind a target antigen.

In one aspect, the present invention provides a stabilized formulation including at least one A β binding polypeptide, at least one tonicity agent, wherein the tonicity agent is present in an amount sufficient to render the formulation suitable for administration,
15 and at least one buffering agent in an amount sufficient to maintain a physiologically suitable pH. The formulation can be a lyophilized or a liquid formulation. Some formulations include at least one antioxidant, such as, for example, an amino acid antioxidant, such as, for example, methionine. In some formulations, the tonicity agent is mannitol or NaCl. In some formulations, at least one buffering agent is succinate,
20 sodium phosphate, or an amino acid such as histidine. Preferred formulations also include at least one stabilizer such as, for example, polysorbate 80. In some formulations, the stabilizer is polysorbate 80, the antioxidant is methionine, the tonicity agent is mannitol, sorbitol or NaCl, and the buffering agent is histidine. In some formulations, at least one A β binding polypeptide is selected from the group consisting
25 of an anti A β antibody, an anti A β antibody Fv fragment, an anti A β antibody Fab fragment, an anti A β antibody Fab'(2) fragment, an anti A β antibody Fd fragment, a single-chain anti A β antibody (scFv), a single domain anti A β antibody fragment (Dab), a beta-pleated sheet polypeptide including at least one antibody complementarity determining region (CDR) from an anti A β antibody, and a non-globular polypeptide
30 including at least one antibody CDR from an anti A β antibody. In some formulations, at least one A β binding polypeptide is an anti A β antibody, for example, that specifically binds to epitope within residues 1-7, 1-5, 3-7, 3-6, 13-28, 15-24, 16-24, 16-21, 19-22,

33-40, 33-42 of A β , or Fab, Fab'(2) or Fv fragment thereof. Exemplary anti A β antibodies specifically bind to an epitope within residues 1-10 of A β , such as, for example, within residues 1-7, 1-5, 3-7, or 3-6 of A β . Other exemplary anti A β antibodies specifically bind to an epitope within residues 13-28 of A β , such as, for example, within residues 16-21 or 19-22 of A β . Yet other exemplary anti A β antibodies specifically bind to a C terminal epitope of A β such as, for example, 33-40 or 33-42 of A β . Preferred anti A β antibodies include a humanized anti A β antibody, for example, a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, or a humanized 15C11 antibody.

In some formulations, the anti A β antibody binds a discontinuous epitope which includes residues within 1-7 and within 13-28 of A β . In some such formulations, the antibody is bispecific antibody or an antibody made by the process described in International Patent Publication No. WO03/070760. In some such formulations, the epitope is a discontinuous epitope. In preferred formulations, the anti A β antibody is a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, or a humanized 15C11 antibody.

The isotype of the antibody can be IgM, IgG1, IgG2, IgG3, IgG4 or any other pharmaceutically acceptable isotype. In preferred formulations, the isotype is human IgG1 or human IgG4. In some liquid formulations, the concentration of the anti A β antibody is about 0.1 mg/ml to about 60 mg/ml, about 40 mg/ml to about 60 mg/ml, about 50 mg/ml, about 30 mg/ml, about 17 mg/ml to about 23 mg/ml, about 20 mg/ml, about 17 mg/ml, about 10 mg/ml, about 5 mg/ml, about 2 mg/ml, or about 1 mg/ml, preferably about 17 mg/ml to about 23 mg/ml.

In some formulations, at least one tonicity agent is D-mannitol and is present at a concentration of about 1% w/v to about 10% w/v, about 2% w/v to about 6% w/v, or preferably about 4% w/v. In some formulations, at least one buffering agent is histidine and is present at a concentration of about 0.1 mM to about 25 mM, about 5 mM to about 15 mM, preferably about 5 mM or about 10 mM. In other formulations, at least one buffering agent is succinate and is present at a concentration of about 0.1 mM to about 25 mM, such as, for example, at about 10 mM. In some formulations, the antioxidant is

methionine and is present at a concentration of about 0.1 mM to about 25 mM, about 5 mM to about 15 mM, or preferably about 10 mM. In preferred formulations, the stabilizer is polysorbate 80 and is present at a concentration of about 0.001% w/v to about 0.01% w/v, about 0.005% w/v to about 0.01% w/v, or about 0.005% w/v. The
5 formulation can have a pH of about 5 to 7, about 5.5 to about 6.5, about 6.0 to about 6.5, about 6.2, about 6.0, or about 5.5, preferably about 6.0.

A preferred formulation has a pH of about 6.0 to about 6.5 and includes an anti A β antibody that specifically binds to an epitope within residues selected from the group consisting of 1-7, 1-5, 3-7, 3-6, 13-28, 15-24, 16-24, 16-21, 19-22, 33-40 and 33-42 of
10 A β , for example D-mannitol at a concentration of about 2% w/v to about 6%, for example histidine at a concentration of about 0.1 mM to about 25 mM, methionine at a concentration of about 0.1 mM to about 25 mM, and a stabilizer. Preferably, the stabilizer is polysorbate 80 at a concentration of about 0.001% to about 0.01% w/v.

The formulation can be a stabilized liquid polypeptide formulation designed to
15 provide stability and to maintain the biological activity of the incorporated polypeptide. The formulation includes a therapeutically active A β -binding polypeptide and an antioxidant in an amount sufficient to reduce the by-product formation of the polypeptide during storage of the formulation.

Some of the liquid polypeptide formulations are stabilized against the formation
20 of undesired by-products such as high molecular weight polypeptide aggregates, low molecular weight polypeptide degradation products, or mixtures thereof.

In formulations wherein the therapeutic antigen-binding polypeptide is an antibody, the typical high molecular weight aggregates to be minimized are, for example, antibody:antibody complexes, antibody:antibody fragment complexes, antibody
25 fragment:antibody fragment complexes, or mixtures thereof. In general, high molecular weight complexes or by-products have a molecular weight greater than a monomer of the antigen-binding polypeptide, for example, in the case of an IgG antibody, greater than about 150 kD. In such antibody formulations, the typical low molecular weight polypeptide degradation products to be minimized are, for example, complexes
30 consisting of an antibody light chain, an antibody heavy chain, an antibody light chain and heavy chain complex, or mixtures thereof. In general, low molecular weight complexes or by-products have a molecular weight less than that of a monomer of the

antigen-binding polypeptide, for example, in the case of an IgG antibody, less than about 150 kD.

A preferred stabilized formulation of an anti-A β antibody includes methionine as an antioxidant in an amount sufficient to inhibit the formation of undesired by-products, a tonicity agent for example in an amount sufficient to render the formulation suitable for administration, and an amino acid for example or derivative thereof in an amount sufficient to maintain a physiologically suitable pH.

Some formulations are stable when frozen. The formulation can be suitable for administering parenterally, intravenously, intramuscularly, subcutaneously, intracranially, or epidurally, preferably intravenously or subcutaneously. Some formulations can be suitable for targeted delivery to the brain or the spinal fluid of a subject. The formulation can be substantially free of preservatives. Some formulations are stable for at least about 12 months, at least about 18 months, at least about 24 months, or at least about 30 months. Some formulations are stable at about -80°C to about 40°C, at about 0°C to about 25°C, at about 0°C to about 10°C, preferably at about -80°C to about -50°C or at about 2°C to about 8°C.

Some formulations are stable for at least about 12 months at a temperature of above freezing to about 10°C and has a pH of about 5.5 to about 6.5. Such formulation includes at least one A β antibody at a concentration of about 1 mg/ml to about 30 mg/ml, mannitol at a concentration of about 4% w/v or NaCl at a concentration of about 150 mM, histidine or succinate at a concentration of about 5 mM to about 10 mM, and 10 mM methionine. One such formulation has a pH of about 6.0, about 1 mg/ml A β antibody, about 10 mM histidine and about 4% w/v mannitol. Other formulations are stable for at least about 24 months at a temperature of about 2°C to 8°C, and include polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v. Some of such formulations have a pH of about 6.0 to about 6.5 and include about 10 mM histidine, about 4% w/v mannitol and about 1 mg/ml, about 2 mg/ml or about 5 mg/ml A β antibody. Other such formulations include about 10 mM histidine, about 4% w/v mannitol, about 0.005% w/v polysorbate 80 and about 10 mg/ml, about 20 mg/ml or 30 mg/ml A β antibody, preferably at a pH of about 6.0 to about 6.2.

The anti A β antibody in such formulations is preferably a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266

antibody, a humanized 12A11 antibody, or a humanized 15C11 antibody. One such formulation has a pH of about 6.0 to 6.5 and includes about 10 mM histidine, about 4% w/v mannitol and about 2 mg/ml to about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, and a humanized 12A11 antibody. Another such formulation has a pH of about 6.0 to 6.5 and includes about 10 mM histidine, about 150 mM NaCl and about 2 mg/ml to about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 12B4 antibody and a humanized 12A11 antibody. Yet another such formulation has a pH of about 6.0 to 6.5 and includes about 10 mM histidine, about 4% w/v mannitol and about 2 mg/ml to about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 266 antibody and a humanized 15C11 antibody.

A preferred formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 5.5 to about 6.5, and includes about 2 mg/ml to about 23 mg/ml, preferably about 17 mg/ml to about 23 mg/ml, of a humanized 3D6 antibody, about 10 mM histidine and about 10 mM methionine. Preferably, the formulation further includes about 4% w/v mannitol. The formulation preferably includes polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v, more preferably about 0.005% w/v polysorbate 80. In such formulations, the humanized 3D6 antibody can be present at a concentration of about 20 mg/ml to about 23 mg/ml.

Another formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 5.5 to about 6.5, and includes about 2 mg/ml to about 23 mg/ml of a humanized 3D6 antibody, about 10 mM succinate, about 10 mM methionine, about 4% w/v mannitol and about 0.005% w/v polysorbate 80. In some of such formulations, the humanized 3D6 antibody concentration is present at a concentration of about 17 mg/ml to about 23 mg/ml.

Another preferred formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 6.0 to about 6.5, and includes about 2 mg/ml to about 30 mg/ml of a humanized 266 antibody, about 10 mM histidine and about 10 mM methionine. Some of such formulations further include about 4% w/v mannitol. Some of such formulations include polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v, for example, about 0.005% w/v polysorbate 80. In

some of such formulations, the humanized 266 antibody is present at a concentration of about 17 mg/ml to about 23 mg/ml or about 20 mg/ml to about 23 mg/ml.

Yet another formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 6.0 to about 6.5, and includes about 2 mg/ml to about 20 mg/ml of a humanized 266 antibody, about 10 mM succinate, about 10 mM methionine, about 4% w/v mannitol and about 0.005% w/v polysorbate.

Another preferred formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 6.0 to about 6.5, and includes about 2 mg/ml to about 30 mg/ml of a humanized 12A11 antibody, about 10 mM histidine and about 10 mM methionine. Some of such formulations include about 150 mM NaCl. Such formulations can include polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v, such as, for example, about 0.005% w/v polysorbate 80. In some of the formulations, the humanized 12A11 antibody is present at a concentration of about 17 mg/ml to about 23 mg/ml or about 20 mg/ml to about 23 mg/ml.

Yet another formulation is stable for at least about 24 months at a temperature of about 2°C to about 8°C, has a pH of about 6.0 to about 6.5, and includes about 2 mg/ml to about 20 mg/ml of a humanized 12A11 antibody, about 5 mM histidine, about 10 mM methionine, about 4% w/v mannitol and about 0.005% w/v polysorbate 80.

The invention also provides a formulation that is stable when thawed from about -50°C to about -80°C, has a pH of about 6.0 and includes about 40 to about 60 mg/ml of an anti A β antibody, about 1.0 mg/ml to about 2.0 mg/ml histidine, about 1.0 mg/ml to 2.0 mg/ml methionine and about 0.05 mg/ml polysorbate 80. Preferably, mannitol is excluded. Preferably, the A β antibody is a humanized 3D6 antibody or a humanized 266 antibody.

The present invention also provides a liquid formulation including an anti A β antibody, mannitol and histidine. In some of such formulations, the anti A β antibody is present from about 1 mg/ml to about 30 mg/ml. Preferably, the mannitol is present in an amount sufficient to maintain isotonicity of the formulation. Preferably, the histidine is present in an amount sufficient to maintain a physiologically suitable pH. One such formulation includes about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about

10 mM methionine, about 4% mannitol and has a pH of about 6. Another such formulation includes about 30 mg/mL anti A β antibody, about 10 mM succinate, about 10 mM methionine, about 6% mannitol and has a pH of about 6.2. Yet another such formulation includes about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about 5 10 mM methionine, about 4% mannitol, about 0.005% polysorbate 80, and has a pH of about 6. Another such formulation includes about 10 mg/mL anti A β antibody, about 10 mM succinate, about 10 mM methionine, about 10% mannitol, about 0.005% polysorbate 80, and has a pH of about 6.5.

Still another such formulation includes about 5 mg/mL to about 20 mg/mL anti 10 A β antibody, about 5 mM to about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol, about 0.005% polysorbate 80, and has a pH of about 6.0 to about 6.5. Yet another such formulation includes about 5 mg/mL to about 20 mg/mL anti A β antibody, about 5 mM to about 10 mM L-histidine, about 10 mM methionine, about 150 mM NaCl, about 0.005% polysorbate 80, and has a pH of about 6.0 to about 6.5.

15 The present invention also provides a formulation suitable for intravenous administration that includes about 20 mg/mL of an anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol and has a pH of about 6. Preferably, such formulation includes about 0.005% polysorbate 80.

The invention provides a method for increasing the stability of an antigen- 20 binding polypeptide, for example, an antibody, in a liquid pharmaceutical formulation, where the polypeptide would otherwise exhibit by-product formation during storage in a liquid formulation. Accordingly, the method comprises incorporating into the formulation an anti-oxidant, for example, methionine or an analog thereof, in an amount sufficient to reduce the amount of by-product formation.

25 The present invention also provides a method for maintaining the stability of a humanized anti A β antibody formulation to be stored at a temperature of about -50°C to about -80°C followed by storage at a temperature of about 2°C to about 8°C , comprising (i) combining about 40 mg/ml to about 60 mg/ml humanized anti A β antibody, about 1 mg/ml to about 2 mg/ml L-histidine, about 1 mg/ml to about 2 mg/ml methionine and 30 about 0.05 mg/ml polysorbate 80; (ii) adjusting the pH to about 6.0; (iii) filtering into a cryovessel and freezing; (iv) thawing; (v) adding mannitol or NaCl and diluent in amounts sufficient to result in a final concentration of about 4% mannitol or about 150

mM NaCl, about 2 mg/ml to about 20 mg/ml humanized anti A β antibody; about 5 mM to about 10 mM histidine; about 10 mM methionine and about 0.005% polysorbate 80; (vi) filtering; (vii) transferring to a glass vial and sealing; and (viii) storing at a temperature of about 2°C to about 8°C.

5 The present invention also provides a kit including a container with a formulation described herein and instructions for use.

 The present invention also provides a pharmaceutical unit dosage form, including a formulation of about 10 mg to about 250 mg of an anti A β antibody, about 4% mannitol or about 150 mM NaCl, about 5 mM to about 10 mM histidine or succinate,
10 and about 10 mM methionine. Some of such pharmaceutical unit dosage forms include about 0.001% to about 0.1% of polysorbate 80. Some of such pharmaceutical unit dosage forms include about 40 mg to about 60 mg, about 60 mg to about 80 mg, about 80 mg to about 120 mg, about 120 mg to about 160 mg, or about 160 mg to about 240 mg of the anti A β antibody. Some of such formulations can be maintained in a glass vial
15 at a temperature of about 2°C to about 8°C prior to administration to a patient.

 In addition, the present invention provides a therapeutic product including a glass vial with a formulation including about 10 mg to about 250 mg of a humanized anti A β antibody, about 4% mannitol or about 150 mM NaCl, about 5 mM to about 10 mM histidine, and about 10 mM methionine. Some of such therapeutic products further
20 include a labeling for use including instructions to use the appropriate volume necessary to achieve a dose of about 0.15 mg/kg to about 5 mg/kg in a patient. Typically, the vial is a 1 mL, a 2 mL, a 5 mL, a 10 mL, a 25 mL or a 50 mL vial. The dose of some of such therapeutic products is about 0.5 mg/kg to about 3 mg/kg, preferably about 1 mg/kg to about 2 mg/kg. In some such therapeutic products, the anti A β antibody concentration is
25 about 10 mg/ml to about 60 mg/ml, preferably about 20 mg/ml. The therapeutic product preferably includes about 0.005% polysorbate 80. The formulation of some such therapeutic products is for subcutaneous administration or intravenous administration.

 The present invention also provides a method for prophylactically or therapeutically treating a disease characterized by A β deposits that includes
30 intravenously or subcutaneously administering a pharmaceutical unit dosage as described herein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a schematic representation of the predicted structure of an IgG antibody and approximate positions of intra- and inter-chain disulfide bonds, glycosylation sites (hexagonal symbol), complementarity determining regions (CDRs), framework regions (shaded), and constant regions.

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Figure 2 shows the complete amino acid sequences of the humanized 3D6 version 2 (hu3D6.v2) anti A β antibody light and heavy chains, SEQ ID NO1 and SEQ ID NO:2, respectively. Light chain complementarity determining regions (CDR), *i.e.*, CDR1, CDR2, and CDR3 are, respectively, at residue positions 24-39, 55-61, and 94-102 (upper panel). Heavy chain complementarity determining regions (CDR), *i.e.*, CDR1, CDR2, and CDR3 are, respectively, at residue positions 40-44, 50-65, and 99-108 (lower panel). Predicted intramolecular disulfide bonds are illustrated by connections of the cysteine residues involved. Cysteines expected to form intermolecular disulfide bonds are underlined and the connectivity indicated. The N-linked glycosylation consensus site of the antibody heavy chain is indicated in italics at residue positions 299-301 (lower panel). The predicted heavy chain C-terminal lysine is shown in parenthesis.

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Figure 3 graphically depicts the shelf life predictions for antibody formulations (with and without polysorbate 80 (PS80)) made in accordance with the present invention and stored at 5°C.

Figure 4 graphically depicts the shelf life predictions for antibody formulations (with and without PS80) made in accordance with the present invention and stored at 25°C.

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Figure 5 graphically depicts the shelf life predictions for antibody formulations (with and without PS80) made in accordance with the present invention and stored at 40°C.

Figure 6 graphically depicts the degradation predictions of formulations with PS80 made in accordance with the present invention and stored at 5°C.

Figure 7 graphically depicts the size exclusion chromatography (SEC) analysis of formulations with PS80 made in accordance with the present invention, stored at 5°C, and reprocessed to minimize assay variability.

Figure 8 graphically depicts the degradation predictions of formulations without PS80 made in accordance with the present invention and stored at 5°C.

Figure 9 depicts a chromatogram which indicates that the presence of PS80 shifts the by-products found within the stabilized polypeptide formulation from a high molecular weight species to a low molecular weight species without changing the monomer antibody profile.

Figure 10 graphically depicts the inhibition of the formation of undesired by-products in a polypeptide formulation comprising IgG4, in particular, high molecular weight polypeptide aggregates, upon the addition of an antioxidant such as free methionine.

Figure 11 graphically depicts the inhibition of the formation of undesired by-products in a polypeptide formulation comprising IgG2, in particular, high molecular weight polypeptide aggregates, upon the addition of an antioxidant such as free methionine.

DETAILED DESCRIPTION OF THE INVENTION

In order to provide a clear understanding of the specification and claims, the following definitions are conveniently provided below.

As used herein, the term “amyloidogenic disease” includes any disease associated with (or caused by) the formation or deposition of insoluble amyloid fibrils. Exemplary amyloidogenic diseases include, but are not limited to systemic amyloidosis, Alzheimer’s disease, mature onset diabetes, Parkinson’s disease, Huntington’s disease, fronto-temporal dementia, and the prion-related transmissible spongiform encephalopathies (kuru and Creutzfeldt-Jacob disease in humans and scrapie and BSE in sheep and cattle, respectively). Different amyloidogenic diseases are defined or characterized by the nature of the polypeptide component of the fibrils deposited. For example, in subjects or patients having Alzheimer’s disease, β -amyloid protein (for example, wild-type, variant, or truncated β -amyloid protein) is the characterizing polypeptide component of the amyloid deposit. Accordingly, Alzheimer’s disease is an

example of a “disease characterized by deposits of A β ” or a “disease associated with deposits of A β ”, for example, in the brain of a subject or patient.

The terms “ β -amyloid protein”, “ β -amyloid peptide”, “ β -amyloid”, “A β ” and “A β peptide” are used interchangeably herein.

5 The term “A β binding polypeptide” includes polypeptides capable of specifically binding to A β peptide(s) or to epitope(s) within said A β peptides. Typically, A β binding polypeptides comprise at least a functional portion of an immunoglobulin or immunoglobulin-like domain, for example, a receptor that comprises one or more variability regions or complementarity determining regions (CDRs) which impart a
10 specific binding characteristic to the polypeptide. Preferred antigen-binding polypeptides include antibodies, for example, IgM, IgG1, IgG2, IgG3, or IgG4.

 The term “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules (molecules that contain an antigen binding site which specifically binds an antigen), including monoclonal antibodies (including full
15 length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (for example, bispecific antibodies), chimeric antibodies, CDR-grafted antibodies, humanized antibodies, human antibodies, and single chain antibodies (scFvs). The term “monoclonal antibody” or “monoclonal antibody composition”, as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding
20 site capable of recognizing and binding to a particular epitope of a target antigen, for example, an epitope(s) of A β . A monoclonal antibody composition thus typically displays a single binding specificity and affinity for a particular target antigen with which it immunoreacts. The term “single-chain antibody” refers to a protein having a two-polypeptide chain structure consisting of a heavy and a light chain, said chains being
25 stabilized, for example, by interchain peptide linkers, which has the ability to specifically bind antigen. Techniques for producing single chain antibodies specific to target antigen are described, for example, in U.S. Patent No. 4,946,778. The term “antibody fragment” includes F(ab')₂ fragments, Fab fragments, Fab' fragments, Fd fragments, Fv fragments, and single domain antibody fragments (DABs). Immunologically active portions of
30 immunoglobulins include, for example, F(ab) and F(ab')₂ fragments. Methods for the construction of Fab fragments are described, for example, Huse, et al. (1989) Science 246:1275 1281). Other antibody fragments may be produced by techniques known in the

art including, but not limited to: (i) an F(ab')₂ fragment produced by pepsin digestion of an antibody molecule; (ii) a Fab fragment generated by reducing the disulfide bridges of an F(ab')₂ fragment; (iii) a Fab' fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) Fv fragments. Various fragments
5 can also be produced by art-recognized recombinant engineering techniques. Non-human antibodies can be "humanized" by techniques described, for example, in U.S. Patent No. 5,225,539. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity

10 The term "domain" refers to a globular region of a heavy or light chain polypeptide comprising an immunoglobulin fold. The immunoglobulin fold is comprised of β -pleated sheet secondary structure and includes a single disulfide bond. Domains are further referred to herein as "constant" or "variable", based on the relative lack of sequence variation within the domains of various class members in the case of a
15 "constant" domain, or the significant variation within the domains of various class members in the case of a "variable" domain. Antibody or polypeptide "domains" are often referred to interchangeably in the art as antibody or polypeptide "regions". The "constant" domains of an antibody light chain are referred to interchangeably as "light chain constant regions", "light chain constant domains", "CL" regions or "CL" domains.
20 The "constant" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "CH" regions or "CH" domains). The "variable" domains of an antibody light chain are referred to interchangeably as "light chain variable regions", "light chain variable domains", "VL" regions or "VL" domains). The "variable" domains of an antibody heavy chain are
25 referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "VH" regions or "VH" domains).

The term "region" can also refer to a part or portion of an antibody chain or antibody chain domain (for example, a part or portion of a heavy or light chain or a part or portion of a constant or variable domain, as defined herein), as well as more discrete
30 parts or portions of said chains or domains. For example, light and heavy chains or light and heavy chain variable domains include "complementarity determining regions" or "CDRs" interspersed among "framework regions" or "FRs", as defined herein.

The term “anti A β antibody” includes antibodies (and fragments thereof) that are capable of binding epitopes(s) of the A β peptide. Anti A β antibodies include, for example, those antibodies described in U.S. Patent Publication No. 20030165496A1, U.S. Patent Publication No. 20040087777A1, International Patent Publication No. WO02/46237A3, and International Patent Publication No. WO04/080419A2. Other anti A β antibodies are described in, for example, International Patent Publication Nos. WO03/077858A2 and WO04/108895A2, both entitled “Humanized Antibodies that Recognize Beta Amyloid Peptide”, International Patent Publication No. WO03/016466A2, entitled “Anti-A β Antibodies”, International Patent Publication No. WO0162801A2, entitled “Humanized Antibodies that Sequester Amyloid Beta Peptide”, and International Patent Publication No. WO02/088306A2, entitled “Humanized Antibodies” and International Patent Publication No. WO03/070760A2, entitled “Anti-A β Antibodies and Their Use.”

The term “fragment” refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. Fragments can be obtained *via* chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. Exemplary fragments include Fab, Fab', F(ab')₂, Fabc and/or Fv fragments. The term “antigen-binding fragment” refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with the intact antibody from which they were derived for specific antigen binding.

The term “conformation” refers to the tertiary structure of a protein or polypeptide, such as, for example, an antibody, antibody chain, domain or region thereof. For example, the phrase “light (or heavy) chain conformation” refers to the tertiary structure of a light (or heavy) chain variable region, and the phrase “antibody conformation” or “antibody fragment conformation” refers to the tertiary structure of an antibody or fragment thereof.

The term “specific binding” of an antibody means that the antibody exhibits appreciable affinity for a particular antigen or epitope and, generally, does not exhibit significant cross-reactivity. In exemplary embodiments, the antibody exhibits no cross-reactivity (for example, does not cross-react with non-A β peptides or with remote or distant epitopes on A β). “Appreciable” or preferred binding includes binding with an affinity of at least 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M, or 10⁻¹⁰ M. Affinities greater than 10⁻⁷ M,

preferably greater than 10^{-8} M are more preferred. Values intermediate of those set forth herein are also intended to be within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities, for example, 10^{-6} to 10^{-10} M, preferably 10^{-7} to 10^{-10} M, more preferably 10^{-8} to 10^{-10} M. An antibody that “does not
5 exhibit significant cross-reactivity” is one that will not appreciably bind to an undesirable entity (for example, an undesirable protein, polypeptide, or peptide). For example, an antibody that specifically binds to A β will appreciably bind A β but will not significantly react with non-A β proteins or peptides (for example, non-A β proteins or peptides included in plaques). An antibody specific for a particular epitope will, for
10 example, not significantly cross-react with remote or different epitopes on the same protein or peptide. Specific binding can be determined according to any art-recognized means for determining such binding. Preferably, specific binding is determined according to Scatchard analysis and/or competitive binding assays.

Binding fragments are produced by recombinant DNA techniques, or by
15 enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', F(ab')₂, Fabc, Fv, single chains, and single-chain antibodies. Other than “bispecific” or “bifunctional” immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A “bispecific” or “bifunctional antibody” is an artificial hybrid antibody having two different heavy/light
20 chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, for example, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

An “antigen” is a molecule (for example, a protein, polypeptide, peptide,
25 carbohydrate, or small molecule) containing an antigenic determinant to which an antibody specifically binds.

The term “epitope” or “antigenic determinant” refers to a site on an antigen to which an immunoglobulin or antibody (or antigen binding fragment thereof) specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous
30 amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with

denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, for example, *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, G. E. Morris, Ed. (1996).

The term “stabilized formulation” or “stabilized liquid polypeptide formulation” includes formulations in which the polypeptide therein essentially retains its physical and chemical identity and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are described herein (reviewed in, Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993)). Stability can be measured at a selected temperature for a selected time period. For rapid testing, the formulation may be kept at a higher or “accelerated” temperature, for example, 40°C for 2 weeks to 1 month or more at which time stability is measured. In exemplary embodiments, the formulation is refractory to the formation of by-products of the component polypeptide, for example, high molecular weight aggregation products, low molecular weight degradation or fragmentation products, or mixtures thereof. The term “stability” refers to the length of time over which a molecular species such as an antibody retains its original chemical identity, for example, primary, secondary, and/or tertiary structure.

The term “by-product” includes undesired products, which detract, or diminish the proportion of therapeutic polypeptide in a given formulation. Typical by-products include aggregates of the therapeutic polypeptide, fragments of the therapeutic polypeptide (for example, produced by degradation of the polypeptide by deamidation or hydrolysis), or mixtures thereof.

The term “high molecular weight polypeptide aggregates” includes aggregates of the therapeutic polypeptide, fragments of the therapeutic polypeptide (for example, produced by degradation of the polypeptide by, for example, hydrolysis), or mixtures thereof, that then aggregate. Typically, high molecular weight aggregates are complexes which have a molecular weight which is greater than the therapeutic monomer polypeptide. In the case of an antibody, for example, an IgG antibody, such aggregates are greater than about 150 kD. However, in the case of other therapeutic polypeptides,

for example, single-chain antibodies, which typically have a molecular weight of 25 kD, such aggregates would have a molecular weight greater than about 25 kD.

The term “low molecular weight polypeptide degradation product” includes, for example, fragments of the therapeutic polypeptide, for example, brought about by
5 deamidation or hydrolysis. Typically, low molecular weight degradation products are complexes which have a molecular weight which is less than the therapeutic monomer polypeptide. In the case of an antibody, for example, an IgG antibody, such degradation products are less than about 150 kD. However, in the case of other therapeutic
10 polypeptides, for example, single-chain antibodies, which typically have a molecular weight of 25 kD, such aggregates would have a molecular weight less than about 25 kD.

The term “administration route” includes art recognized administration routes for delivering a therapeutic polypeptide such as, for example, parenterally, intravenously, intramuscularly, subcutaneously, intracranially, or epidurally. For the administration of a therapeutic polypeptide for the treatment of a neurodegenerative disease, intravenous,
15 epidural, or intracranial routes, may be desired.

The term “treatment” as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal,
20 alleviate, delay, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

The term “effective dose” or “effective dosage” is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term “therapeutically effective dose” is defined as an amount sufficient to cure or at least
25 partially arrest the disease and its complications in a patient already suffering from the disease. Amounts effective for this use will depend upon the severity of the infection and the general state of the patient’s own immune system.

The term “patient” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

30 The term “dosage unit form” (or “unit dosage form”) as used herein refers to a physically discrete unit suitable as unitary dosages for the patient to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier,

diluent, or excipient. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of patients.

5 Actual dosage levels of the active ingredient (for example A β polypeptides) in the formulations of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors
10 including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the
15 patient being treated, and like factors well known in the medical arts.

The term “diluent” as used herein refers to a solution suitable for altering or achieving an exemplary or appropriate concentration or concentrations as described herein.

20 *OVERVIEW*

The present invention provides formulations for A β binding polypeptides, in particular, anti A β antibodies, as well as portions and/or fragments thereof. In certain aspects, the invention provides stabilized liquid polypeptide formulations for therapeutic use. In particular, the invention provides for the stabilization of A β binding polypeptides, for example, antibodies, and antigen-binding fragments thereof, for the use in treating amyloidogenic diseases and /or disorders. In particular, the invention provides formulations that are stabilized such that the active therapeutic polypeptide is stable over an extended period of time and can be administered through a variety of administration routes. This is especially critical for those A β binding polypeptides (for example, antibodies) destined for use in the treatment of amyloidogenic diseases and /or disorders. In other aspects, the invention provides a uniquely stable antibody formulation that, for example, is stable to various stresses such as freezing, lyophilization, heat and/or reconstitution. Moreover, exemplary formulations of the

present invention are capable of maintaining the stability, biological activity, purity and quality of the antibody over an extended period of time (for example, a year or more during which time the formulation is stored) and even at unfavorable temperatures. In addition, exemplary formulations of the present invention are suitable for administration
5 to a subject or patient (for example, intravenous administration to a subject or patient), for example, a human having or predicted to have an amyloidogenic disease or disorder.

FORMULATIONS

In one aspect, the present invention provides a stabilized formulation including
10 an A β binding polypeptide, a tonicity agent, where the tonicity agent is present in an amount sufficient to render the stabilized formulation suitable for intravenous infusion, and an amino acid or derivative thereof, where the amino acid or derivative thereof is present in an amount sufficient to maintain a physiologically suitable pH. In an exemplary embodiment, the present invention provides a stabilized formulation
15 including an anti A β antibody, mannitol and histidine.

In one embodiment, the present invention provides a stabilized formulation including an A β binding polypeptide, a tonicity agent, wherein the tonicity agent is present in an amount sufficient to render the formulation suitable for intravenous infusion, and an amino acid or derivative thereof, where the amino acid or derivative
20 thereof is present in an amount sufficient to maintain a physiologically suitable pH. In an exemplary embodiment, the tonicity agent is mannitol. In another exemplary embodiment, the amino acid is histidine.

In another aspect, the present invention provides a stabilized formulation including an A β binding polypeptide. A β binding polypeptides suitable for stabilization
25 in a formulation of the invention include antibodies and fragments thereof, and in particular, antibodies capable of binding a therapeutic target involved in amyloidogenic disease or disorder. Accordingly, the therapeutic polypeptides are stabilized according to the invention to avoid the formation of by-products, typically high molecular weight aggregates, low molecule weight degradation fragments, or a mixture thereof, by the
30 addition of an antioxidant in a sufficient amount so as to inhibit the formation of such by-products. Antioxidant agents include methionine and analogs thereof, at concentrations sufficient to obtain the desired inhibition of undesired by-products as discussed below. Optionally, the stabilized polypeptide formulations of the invention

further comprise a tonicity agent, where the tonicity agent is present in an amount sufficient to render the stabilized formulation suitable for several different routes of administration, for example, intravenous infusion, and an amino acid or derivative thereof, where the amino acid or derivative thereof is present in an amount sufficient to
5 maintain a physiologically suitable pH. In an exemplary embodiment, the present invention provides a stabilized formulation including an anti A β antibody, methionine, mannitol and histidine.

In one embodiment, the present invention provides a stabilized liquid formulation including a therapeutically active A β binding polypeptide, wherein the
10 polypeptide is capable of by-product formation during storage and an antioxidant, where the antioxidant is present in an amount sufficient to reduce by-product formation during storage of the formulation. In an exemplary embodiment, the anti-oxidant is methionine or an analog thereof.

In some embodiments of the invention, the A β binding polypeptide is selected
15 from the group consisting of an antibody, an antibody Fv fragment, an antibody Fab fragment, an antibody Fab'(2) fragment, an antibody Fd fragment, a single-chain antibody (scFv), a single domain antibody fragment (Dab), a beta-pleated sheet polypeptide including at least one antibody complementarity determining region (CDR), and a non-globular polypeptide including at least one antibody complementarity
20 determining region. In exemplary embodiments of the invention, the A β binding polypeptide is present from about 0.1 mg/ml to about 60 mg/ml. In other exemplary embodiments, formulations of the present invention include A β binding polypeptide at about 30 mg/ml. In yet other exemplary embodiments, formulations of the present invention include A β binding polypeptide at about 20 mg/ml. In further exemplary
25 embodiments, formulations of the invention include A β binding polypeptide at about 17 mg/ml.

In exemplary embodiments of the invention, the A β binding polypeptide is an anti A β antibody. In some embodiments of the present invention, the anti A β antibody is selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5
30 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, and a humanized 15C11 antibody. In exemplary embodiments of the present invention, the anti A β antibody binds to an epitope including A β amino acid residues selected from the group consisting of 1-7, 1-5, 3-7, 3-6, 13-28, 16-21, 19-22, 33-40, and

33-42. In some embodiments of the present invention, the anti A β antibody is of a subtype selected from the group consisting of human IgG1, IgG2, IgG3, and IgG4. In a particular embodiment of the present invention, the anti A β antibody is of a human IgG1 subtype.

5 The A β polypeptide may be capable of forming a by-product selected from the group consisting of a high molecular weight polypeptide aggregate, a low molecular weight polypeptide degradation product, and combinations thereof. The high molecular weight aggregates may include antibody:antibody complexes, antibody:antibody fragment complexes, antibody fragment:antibody fragment complexes, and combinations
10 thereof. The low molecular weight polypeptide degradation product may include an antibody light chain, an antibody heavy chain, an antibody light chain and heavy chain complex, an antibody fragment, and combinations thereof.

 In one embodiment of the present invention, a liquid formulation according to the present invention includes an A β binding polypeptide, mannitol and histidine. In an
15 exemplary embodiment of the present invention, the A β binding polypeptide is an anti A β antibody. In some exemplary embodiments of the present invention, the anti A β antibody is selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, and a humanized 15C11 antibody. In other exemplary
20 embodiments of the present invention, the anti A β antibody binds to an epitope including A β amino acid residues selected from the group consisting of 1-7, 1-5, 3-7, 3-6, 13-28, 16-21, 19-22, 33-40, and 33-42. In some embodiments of the present invention, the antibody is of a subtype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4. In a particular embodiment of the present invention, the antibody is of an IgG1
25 subtype.

 In exemplary embodiments of the present invention, the anti A β antibody is present from about 0.1 mg/ml to about 200 mg/ml. In other exemplary embodiments of the present invention, the anti A β antibody is present at about 20 mg/ml.

 In some embodiments of the present invention, formulations of the present
30 invention include mannitol in an amount sufficient to maintain isotonicity of the formulation. In exemplary embodiments of the present invention, mannitol is present from about 2% w/v to about 10% w/v. In other exemplary embodiments of the present invention, mannitol is present at about 4% w/v. In yet other exemplary embodiments,

mannitol is present at about 6% w/v. In further exemplary embodiments, mannitol is present at about 10% w/v.

In some embodiments of the present invention, formulations of the present invention include histidine in an amount sufficient to maintain a physiologically suitable pH. In exemplary embodiments of the present invention, histidine is present from about 0.1 mM to about 25 mM. In other exemplary embodiments, histidine is present at about 10 mM.

In one embodiment of the present invention, formulations of the present invention include succinate from about 0.1 mM to about 25 mM. In an exemplary embodiment of the present invention, succinate is present at about 10 mM.

In some embodiments of the present invention, formulations of the present invention further include an anti-oxidant. In exemplary embodiments, the anti-oxidant is methionine or an analog thereof. In one embodiment of the present invention, the methionine or analog is present at about 0.1 mM to about 25 mM. In another embodiment, the methionine or analog is present at about 10 mM.

In some embodiments of the invention, the formulation further includes a stabilizer. In exemplary embodiments of the present invention, the stabilizer is polysorbate 80. In some embodiments, the polysorbate 80 is present from about 0.001% w/v to about 0.01% w/v. In other embodiments, the polysorbate 80 is present at about 0.005% w/v. In yet other embodiments of the present invention, the polysorbate 80 is present at about 0.01% w/v.

In some embodiments of the invention, the formulation has a pH of about 5 to about 7. In exemplary embodiments of the present invention, the formulation has a pH of about 5.5. In another exemplary embodiment, the formulation has a pH of about 6.0. In yet another exemplary embodiment, the formulation has a pH of about 6.2. In further exemplary embodiments, the formulation has a pH of about 6.5.

In some embodiments, the formulation is stable to freezing. In other embodiments of the present invention, the formulation is suitable for intravenous administration. In an exemplary embodiment of the present invention, the formulation is suitable for intramuscular or subcutaneous administration. In an exemplary embodiment, the formulation is suitable for delivery to the brain of a subject.

In some embodiments of the present invention, the formulation is suitable for delivery to the spinal fluid of a subject. In other embodiments, the formulation is substantially free of preservatives.

In some embodiments of the present invention, the formulation is stable for at least about 12 months. In some embodiments, the formulation is stable for at least about 18 months. In some embodiments of the present invention, the formulation is stable for at least about 24 months. In some embodiments of the present invention, the formulation is stable for at least about 30 months.

In exemplary embodiments of the present invention, the formulation is stable from about -80°C to about 40°C. In some exemplary embodiments, the formulation is stable from about 0°C to about 25°C. Preferably, the formulation is stable from about 2°C to about 8°C.

In a particular embodiment of the present invention, a formulation suitable for intravenous administration includes about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol and has a pH of about 6. In another particular embodiment, a formulation suitable for intravenous administration includes about 30 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 6% mannitol and has a pH of about 6.2. A preferred formulation suitable for intravenous administration includes about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol, about 0.005% polysorbate 80, and has a pH of about 6. In a further exemplary embodiment of the present invention, a formulation suitable for intravenous administration includes about 10 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 10% mannitol, about 0.005% polysorbate 80, and has a pH of about 6.5.

In some embodiments of the foregoing formulations according to the present invention, the anti A β antibody is selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, and a humanized 15C11 antibody. In exemplary embodiments, the anti A β antibody binds to an epitope within amino acid residues selected from the group consisting of 1-7, 1-5, 3-7, 3-6, 13-28, 16-21, 19-22, 33-40, and 33-42 of A β . In some formulations, the anti A β antibody binds a discontinuous epitope which includes residues within 1-7 within 13-28 of A β . In some such formulations, the antibody is a bispecific antibody or an antibody made by the

process described in International Patent Publication No. WO03/070760. In some such formulations, the epitope is a discontinuous epitope.

In another aspect of the present invention, a pharmaceutical unit dosage form includes an effective amount of the formulation of any of the foregoing embodiments for
5 treating disease in a patient via administration of the dosage form to the patient. In an exemplary embodiment, the pharmaceutical unit dosage form is a container containing a formulation according to the present invention. In an exemplary embodiment, the container is a vial containing about 1 mg to about 2000 mg of the A β binding polypeptide. In another exemplary embodiment, the vial contains about 50 mg to about
10 1500 mg of the A β binding polypeptide. In a further exemplary embodiment, the vial contains about 5 mg to about 50 mg of the A β binding polypeptide.

In exemplary embodiments, the vial has a volume of about 2 to about 100 ml. In yet other embodiments, the vial has a volume of about 2 to about 10 ml.

In some embodiments, a pharmaceutical unit dosage form according to the
15 present invention is suitable for intravenous infusion to a patient.

Also described herein are kits including a pharmaceutical unit dosage form, as described herein, and instructions for use. In one embodiment of the present invention, a container including the pharmaceutical unit dosage form of is a container labeled for use. In an exemplary embodiment, the container is labeled for prophylactic use. In another
20 exemplary embodiment, the container is labeled for therapeutic use.

The present invention provides a method for increasing the stability of an A β binding polypeptide in a liquid pharmaceutical formulation, where the polypeptide exhibits by-product formation during storage in a liquid formulation, which method includes incorporating into the formulation an anti-oxidant in an amount sufficient to
25 reduce the amount of by-product formation of the polypeptide. In exemplary embodiments, the A β binding polypeptide component is selected from the group consisting of an antibody, an antibody Fv fragment, an antibody Fab fragment, an antibody Fab'(2) fragment, an antibody Fd fragment, a single-chain antibody (scFv), a single domain antibody fragment (Dab), a beta-pleated sheet polypeptide including at
30 least one antibody complementarity determining region (CDR), and a non-globular polypeptide including at least one antibody complementarity determining region. In one embodiment, the by-product is selected from the group consisting of a high molecular weight polypeptide aggregate, a low molecular weight polypeptide degradation product,

and combinations thereof. In another embodiment, the antioxidant is selected from the group consisting of methionine and an analog thereof.

In some embodiments, a method for preparing a formulation according to any of the foregoing embodiments of the present invention includes combining the excipients of the formulation. In an exemplary embodiment, a method for preparing the formulation according to any of the foregoing embodiments includes combining the A β binding polypeptide with one or more diluents, where the one or more diluents include the excipients of the formulation.

In an exemplary embodiment, a method for preparing a pharmaceutical unit dosage form includes combining the formulation of any of foregoing embodiments in a suitable container. In another exemplary embodiment, a method for preparing the formulation of any of the foregoing embodiments includes combining a solution including the A β binding polypeptide and a least a portion of the excipients of the formulation with a diluent including the remainder of the excipients.

Polypeptides for use in the Stabilized Formulations of the Invention

The polypeptide to be formulated according to the invention as described herein is prepared using techniques which are well established in the art and include, for example, synthetic techniques (such as recombinant techniques and peptide synthesis or a combination of these techniques), or may be isolated from an endogenous source of the polypeptide. In certain embodiments of the invention, the polypeptide of choice is an antigen-binding polypeptide, more preferably, an antibody, and in particular, an anti-A β antibody. Techniques for the production of an antigen-binding polypeptide, and in particular, antibodies, are described below.

Polyclonal Antibodies

Polyclonal antibodies can be prepared by immunizing a suitable subject with an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized target antigen. If desired, the antibody molecules directed against the target antigen can be isolated from the mammal (for example, from the blood) and further purified by well known techniques, such as protein A Sepharose chromatography to obtain the antibody, for example, IgG, fraction. At an appropriate time after

immunization, for example, when the anti-antigen antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75). For the preparation of chimeric polyclonal antibodies, see Buechler *et al.* U.S. Patent No. 6,420,113.

10 Monoclonal Antibodies

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody (see, for example, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al. Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, 15 *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (for example, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic 20 preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 25 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells 30 producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind a target antigen, for example, A β , using a standard ELISA assay.

Recombinant Antibodies

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (for example, an antibody phage display library) with a target antigen to thereby isolate immunoglobulin library members that bind the target antigen. Kits for generating and screening phage display libraries are commercially available (for example, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

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Chimeric and Humanized Antibodies

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention.

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The term “humanized immunoglobulin” or “humanized antibody” refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain (*i.e.*, at least one humanized light or heavy chain). The term “humanized

immunoglobulin chain” or “humanized antibody chain” (*i.e.*, a “humanized immunoglobulin light chain” or “humanized immunoglobulin heavy chain”) refers to an immunoglobulin or antibody chain (*i.e.*, a light or heavy chain, respectively) having a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) (for example, at least one CDR, preferably two CDRs, more preferably three CDRs) substantially from a non-human immunoglobulin or antibody, and further includes constant regions (for example, at least one constant region or portion thereof, in the case of a light chain, and three constant regions in the case of a heavy chain). The term “humanized variable region” (for example, “humanized light chain variable region” or “humanized heavy chain variable region”) refers to a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) substantially from a non-human immunoglobulin or antibody.

The phrase “substantially from a human immunoglobulin or antibody” or “substantially human” means that, when aligned to a human immunoglobulin or antibody amino sequence for comparison purposes, the region shares at least 80-90%, 90-95%, or 95-99% identity (*i.e.*, local sequence identity) with the human framework or constant region sequence, allowing, for example, for conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like. The introduction of conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like, is often referred to as “optimization” of a humanized antibody or chain. The phrase “substantially from a non-human immunoglobulin or antibody” or “substantially non-human” means having an immunoglobulin or antibody sequence at least 80-95%, preferably at least 90-95%, more preferably, 96%, 97%, 98%, or 99% identical to that of a non-human organism, for example, a non-human mammal.

Accordingly, all regions or residues of a humanized immunoglobulin or antibody, or of a humanized immunoglobulin or antibody chain, except the CDRs, are substantially identical to the corresponding regions or residues of one or more native human immunoglobulin sequences. The term “corresponding region” or “corresponding residue” refers to a region or residue on a second amino acid or nucleotide sequence which occupies the same (*i.e.*, equivalent) position as a region or residue on a first amino

acid or nucleotide sequence, when the first and second sequences are optimally aligned for comparison purposes.

The term "significant identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 50-60% sequence identity, preferably at least 60-70% sequence identity, more preferably at least 70-80% sequence identity, more preferably at least 80-90% sequence identity, even more preferably at least 90-95% sequence identity, and even more preferably at least 95% sequence identity or more (for example, 99% sequence identity or more). The term "substantial identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80-90% sequence identity, preferably at least 90-95% sequence identity, and more preferably at least 95% sequence identity or more (for example, 99% sequence identity or more). For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al.*, *Current Protocols in Molecular Biology*). One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized

parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic sidechains): leu, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

Preferably, humanized immunoglobulins or antibodies bind antigen with an affinity that is within a factor of three, four, or five of that of the corresponding non-humanized antibody. For example, if the nonhumanized antibody has a binding affinity of 10^{-9} M, humanized antibodies will have a binding affinity of at least 3×10^{-8} M, 4×10^{-8} M, 5×10^{-8} M, or 10^{-9} M. When describing the binding properties of an immunoglobulin or antibody chain, the chain can be described based on its ability to “direct antigen (for example, A β) binding”. A chain is said to “direct antigen binding” when it confers upon an intact immunoglobulin or antibody (or antigen binding fragment thereof) a specific binding property or binding affinity. A mutation (for example, a backmutation) is said to substantially affect the ability of a heavy or light chain to direct antigen binding if it affects (for example, decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by at least an order of magnitude compared to that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation. A mutation “does not substantially affect (for example, decrease) the ability of a chain to direct antigen binding” if it affects (for example, decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by only a factor of two, three, or four of that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation.

The term “chimeric immunoglobulin” or antibody refers to an immunoglobulin or antibody whose variable regions derive from a first species and whose constant

regions derive from a second species. Chimeric immunoglobulins or antibodies can be constructed, for example by genetic engineering, from immunoglobulin gene segments belonging to different species. The terms “humanized immunoglobulin” or “humanized antibody” are not intended to encompass chimeric immunoglobulins or antibodies, as defined *infra*. Although humanized immunoglobulins or antibodies are chimeric in their construction (*i.e.*, comprise regions from more than one species of protein), they include additional features (*i.e.*, variable regions comprising donor CDR residues and acceptor framework residues) not found in chimeric immunoglobulins or antibodies, as defined herein.

Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

Human Antibodies from Transgenic Animals and Phage Display

Alternatively, it is now possible to produce transgenic animals (for example, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice results in the production of human antibodies

upon antigen challenge. See, for example, U.S. Patent Nos. 6,150,584; 6,114,598; and 5,770,429.

Fully human antibodies can also be derived from phage-display libraries (Hoogenboom *et al.*, J. Mol. Biol., 227:381 (1991); Marks *et al.*, J. Mol. Biol., 222:581-597 (1991)). Chimeric polyclonal antibodies can also be obtained from phage display libraries (Buechler *et al.* U.S. Patent No. 6,420,113).

Bispecific Antibodies, Antibody Fusion Polypeptides, and Single-Chain Antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes. Such antibodies can be derived from full length antibodies or antibody fragments (for example F(ab)'2 bispecific antibodies). Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of different antibody molecules (see, WO 93/08829 and in Traunecker *et al.*, EMBO J., 10:3655-3659 (1991)).

Bispecific antibodies also include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin or other payload. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

In yet another embodiment, the antibody can be fused, chemically or genetically, to a payload such as a reactive, detectable, or functional moiety, for example, an immunotoxin to produce an antibody fusion polypeptide. Such payloads include, for example, immunotoxins, chemotherapeutics, and radioisotopes, all of which are well-known in the art.

Single chain antibodies are also suitable for stabilization according to the invention. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) with a linker, which allows each variable region to interface with each other and recreate the antigen binding pocket of the parent antibody

from which the VL and VH regions are derived. See Gruber *et al.*, J. Immunol., 152:5368 (1994).

It is understood that any of the foregoing polypeptide molecules, alone or in combination, are suitable for preparation as stabilized formulations according to the invention.

Anti A β Antibodies

Generally, the formulations of the present invention include a variety of antibodies for treating amyloidogenic diseases, in particular, Alzheimer's Disease, by targeting A β peptide.

The terms "A β antibody", "anti A β antibody" and "anti A β " are used interchangeably herein to refer to an antibody that binds to one or more epitopes or antigenic determinants of the human amyloid precursor protein (APP), A β protein, or both. Exemplary epitopes or antigenic determinants can be found within APP, but are preferably found within the A β peptide of APP. Multiple isoforms of APP exist, for example APP⁶⁹⁵, APP⁷⁵¹ and APP⁷⁷⁰. Amino acids within APP are assigned numbers according to the sequence of the APP⁷⁷⁰ isoform (see for example, GenBank Accession No. P05067). Examples of specific isotypes of APP which are currently known to exist in humans are the 695 amino acid polypeptide described by Kang *et al.* (1987) *Nature* 325:733-736 which is designated as the "normal" APP; the 751 amino acid polypeptide described by Ponte *et al.* (1988) *Nature* 331:525-527 (1988) and Tanzi *et al.* (1988) *Nature* 331:528-530; and the 770-amino acid polypeptide described by Kitaguchi *et al.* (1988) *Nature* 331:530-532. As a result of proteolytic processing of APP by different secretase enzymes *in vivo* or *in situ*, A β is found in both a "short form", 40 amino acids in length, and a "long form", ranging from 42-43 amino acids in length. The short form, A β ₄₀, consists of residues 672-711 of APP. The long form, for example, A β ₄₂ or A β ₄₃, consists of residues 672-713 or 672-714, respectively. Part of the hydrophobic domain of APP is found at the carboxy end of A β , and may account for the ability of A β to aggregate, particularly in the case of the long form. A β peptide can be found in, or purified from, the body fluids of humans and other mammals, for example cerebrospinal fluid, including both normal individuals and individuals suffering from amyloidogenic disorders.

The terms “ β -amyloid protein”, “ β -amyloid peptide”, “ β -amyloid”, “A β ” and “A β peptide” are used interchangeably herein. A β peptide (for example, A β 39, A β 40, A β 41, A β 42 and A β 43) is a ~4-kDa internal fragment of 39-43 amino acids of APP. A β 40, for example, consists of residues 672-711 of APP and A β 42 consists of residues 672-713 of APP. A β peptides include peptides resulting from secretase cleavage of APP and synthetic peptides having the same or essentially the same sequence as the cleavage products. A β peptides can be derived from a variety of sources, for example, tissues, cell lines, or body fluids (for example sera or cerebrospinal fluid). For example, an A β can be derived from APP-expressing cells such as Chinese hamster ovary (CHO) cells stably transfected with APP_{717V→F}, as described, for example, in Walsh *et al.*, (2002), *Nature*, 416, pp 535-539. An A β preparation can be derived from tissue sources using methods previously described (*see*, for example, Johnson-Wood *et al.*, (1997), *Proc. Natl. Acad. Sci. USA* 94:1550). Alternatively, A β peptides can be synthesized using methods which are well known to those in the art. See, for example, Fields *et al.*, Synthetic Peptides: A User’s Guide, ed. Grant, W.H. Freeman & Co., New York, NY, 1992, p 77). Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α -amino group protected by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431. Longer peptide antigens can be synthesized using well known recombinant DNA techniques. For example, a polynucleotide encoding the peptide or fusion peptide can be synthesized or molecularly cloned and inserted in a suitable expression vector for the transfection and heterologous expression by a suitable host cell. A β peptide also refers to related A β sequences that results from mutations in the A β region of the normal gene.

Exemplary epitopes or antigenic determinants to which an A β antibody binds can be found within the human amyloid precursor protein (APP), but are preferably found within the A β peptide of APP. Exemplary epitopes or antigenic determinants within A β are located within the N-terminus, central region, or C-terminus of A β . An “N-terminal epitope”, is an epitope or antigenic determinant located within or including the N-terminus of the A β peptide. Exemplary N-terminal epitopes include residues within amino acids 1-10 or 1-12 of A β , preferably from residues 1-3, 1-4, 1-5, 1-6, 1-7, 2-6, 2-7, 3-6, or 3-7 of A β 42. Other exemplary N-terminal epitopes start at residues 1-3 and

end at residues 7-11 of A β . Additional exemplary N-terminal epitopes include residues 2-4, 5, 6, 7 or 8 of A β , residues 3-5, 6, 7, 8 or 9 of A β , or residues 4-7, 8, 9 or 10 of A β 42. "Central epitopes" are epitopes or antigenic determinants comprising residues located within the central or mid-portion of the A β peptide. Exemplary central epitopes include residues within amino acids 13-28 of A β , preferably from residues 14-27, 15-26, 16-25, 17-24, 18-23, or 19-22 of A β . Other exemplary central epitopes include residues within amino acids 16-24, 16-23, 16-22, 16-21, 18-21, 19-21, 19-22, 19-23, or 19-24 of A β . "C-terminal" epitopes or antigenic determinants are located within or including the C-terminus of the A β peptide and include residues within amino acids 33-40, 33-41, or 33-42 of A β . "C-terminal epitopes" are epitopes or antigenic determinants comprising residues located within the C-terminus of the A β peptide (for example, within about amino acids 30-40 or 30-42 of A β . Additional exemplary C-terminal epitopes or antigenic determinants include residues 33-40 or 33-42 of A β .

When an antibody is said to bind to an epitope within specified residues, such as A β 3-7, what is meant is that the antibody specifically binds to a polypeptide containing the specified residues (*i.e.*, A β 3-7 in this an example). Such an antibody does not necessarily contact every residue within A β 3-7. Nor does every single amino acid substitution or deletion within A β 3-7 necessarily significantly affect binding affinity. In various embodiments, an A β antibody is end-specific. As used herein, the term "end-specific" refers to an antibody which specifically binds to the N-terminal or C-terminal residues of an A β peptide but that does not recognize the same residues when present in a longer A β species comprising the residues or in APP. In various embodiments, an A β antibody is "C-terminus-specific." As used herein, the term "C terminus-specific" means that the antibody specifically recognizes a free C-terminus of an A β peptide. Examples of C terminus-specific A β antibodies include those that: recognize an A β peptide ending at residue 40 but do not recognize an A β peptide ending at residue 41, 42, and/or 43; recognize an A β peptide ending at residue 42 but do not recognize an A β peptide ending at residue 40, 41, and/or 43; etc.

In one embodiment, the A β antibody may be a 3D6 antibody or variant thereof, or a 10D5 antibody or variant thereof, both of which are described in U.S. Patent Publication No. 20030165496A1, U.S. Patent Publication No. 20040087777A1, International Patent Publication No. WO02/46237A3 and International Patent

Publication No. WO04/080419A2. Description of 3D6 and 10D5 antibodies can also be found, for example, in International Patent Publication No. WO02/088306A2 and International Patent Publication No. WO02/088307A2. Additional 3D6 antibodies are described in U.S. Patent Application No. 11/303,478 and International Application No. PCT/US05/45614. 3D6 is a monoclonal antibody (mAb) that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 1-5. By comparison, 10D5 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 3-6. A cell line producing the 3D6 monoclonal antibody (RB96 3D6.32.2.4) was deposited with the American Type Culture Collection (ATCC), Manassas, VA 20108, USA on April 8, 2003 under the terms of the Budapest Treaty and has deposit number PTA-5130. A cell line producing the 10D5 monoclonal antibody (RB44 10D5.19.21) was deposited with the ATCC on April 8, 2003 under the terms of the Budapest Treaty and has deposit number PTA-5129.

Exemplary variant 3D6 antibodies are those having, for example, a humanized light chain comprising variable region amino acid sequences set forth as SEQ ID NO:3 or SEQ ID NO:5 and a humanized heavy chain comprising variable region amino acid sequences set forth as SEQ ID NO:4 or SEQ ID NO:6. Other exemplary variant 3D6 antibodies are those having, for example, a humanized light chain amino acid sequence set forth as SEQ ID NO:7 and a humanized heavy chain amino acid sequence set forth as SEQ ID NO:8.

Exemplary variant 10D5 antibodies are those having, for example, a humanized light chain comprising variable region amino acid sequences set forth as SEQ ID NO:9 or SEQ ID NO:11 and a humanized heavy chain comprising variable region amino acid sequences set forth as SEQ ID NO:10 or SEQ ID NO:12. Other exemplary variant 10D5 antibodies are those having, for example, a humanized light chain amino acid sequence set forth as SEQ ID NO:13 and a humanized heavy chain amino acid sequence set forth as SEQ ID NO:14. Such variant antibodies are further described in WO02/088306A2.

In another embodiment, the antibody may be a 12B4 antibody or variant thereof, as described in U.S. Patent Publication No. 20040082762A1 and International Patent Publication No. WO03/077858A2. 12B4 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 3-7.

Exemplary variant 12B4 antibodies are those having, for example, a humanized light chain (or light chain) comprising variable region amino acid sequences set forth as

SEQ ID NO:15 or SEQ ID NO:17 and a humanized heavy chain comprising variable region amino acid sequences set forth as SEQ ID NO:16, SEQ ID NO:18 or SEQ ID NO:19.

In yet another embodiment, the antibody may be a 12A11 antibody or a variant thereof, as described in U.S. Patent Publication No. 20050118651A1, U.S. Patent Application Serial No. 11/303,478, International Patent Publication No. WO04/108895A2, and International Patent Application Serial No. PCT/US05/45614. 12A11 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 3-7. A cell line producing the 12A11 monoclonal antibody was deposited with the ATCC on December 12, 2005 under the terms of the Budapest Treaty and has deposit number ____.

Exemplary variant 12A11 antibodies are those having, for example, a humanized light chain comprising the variable region amino acid sequence set forth as SEQ ID NO:20 and a humanized heavy chain comprising variable region amino acid sequences set forth as SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, or SEQ ID NO:41.

In yet another embodiment, the antibody may be a 6C6 antibody, or a variant thereof, as described in a U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that Recognize Beta Amyloid Peptide." 6C6 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 3-7. A cell line producing the antibody 6C6 was deposited on November 1, 2005, with the ATCC under the terms of the Budapest Treaty and assigned accession number PTA-7200.

In yet another embodiment, the antibody may be a 2H3 antibody as described in U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that Recognize Beta Amyloid Peptide." 2H3 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 2-7.

In yet another embodiment, the antibody may be a 3A3 antibody as described in U.S. Patent Application Serial No. _____. 3A3 is a mAb that specifically binds to an N-terminal epitope located in the human β -amyloid peptide, specifically, residues 3-7.

Cell lines producing the antibodies 2H3 and 3A3, having the ATCC accession
5 numbers _____ and _____, respectively, were deposited on December 12, 2005 under the terms of the Budapest Treaty.

In yet another embodiment, the antibody may be a 15C11 antibody or variant thereof, as described in a U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that
10 Recognize Beta Amyloid Peptide." 15C11 is a mAb that specifically binds to a central epitope located in the human β -amyloid peptide, specifically, residues 19-22. A cell line producing the 15C11 monoclonal antibody was deposited with the ATCC on December 12, 2005 under the terms of the Budapest Treaty and has deposit number _____.

In yet another embodiment, the antibody may be a 266 antibody as described in
15 U.S. Patent Publication No. 20050249725A1, and International Patent Publication No. WO01/62801A2. 266 is a mAb that specifically binds to a central epitope located in the human β -amyloid peptide, specifically, residues 16-24. A cell line producing the 266 monoclonal antibody was deposited with the ATCC on July 20, 2004 under the terms of the Budapest Treaty and has deposit number PTA-6123.

Exemplary variant 266 antibodies are those having, for example, a humanized
20 light chain comprising variable region amino acid sequences set forth as SEQ ID NO:42 or SEQ ID NO:44 and a humanized heavy chain comprising variable region amino acid sequences set forth as SEQ ID NO:43 or SEQ ID NO:45. Other exemplary variant 266 antibodies are those having, for example, a humanized light chain amino acid sequence
25 set forth as SEQ ID NO:46 and a humanized heavy chain amino acid sequence set forth as SEQ ID NO:47. Such variant antibodies are further described in U.S. Patent Publication No. 20050249725A1, and International Patent Publication No. WO01/62801A2.

In yet another embodiment, the antibody may be a 2B1 antibody, or a variant
30 thereof, as described in a U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that Recognize Beta Amyloid Peptide." 2B1 is a mAb that specifically binds to a central epitope located in the human β -amyloid peptide, specifically, residues 19-23.

In yet another embodiment, the antibody may be a 1C2 antibody, or a variant thereof, as described in a U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that Recognize Beta Amyloid Peptide." 1C2 is a mAb that specifically binds to a central epitope located in the human β -amyloid peptide, specifically, residues 16-23.

In yet another embodiment, the antibody may be a 9G8 antibody, or a variant thereof, as described in a U.S. Patent Application No. 11/304,986 and International Patent Application No. PCT/US05/45515 entitled "Humanized Antibodies that Recognize Beta Amyloid Peptide." 9G8 is a mAb that specifically binds to a central epitope located in the human β -amyloid peptide, specifically, residues 16-21.

Cell lines producing the antibodies 2B1, 1C2 and 9G8 were deposited on November 1, 2005, with the ATCC under the terms of the Budapest Treaty and were assigned accession numbers PTA-7202, PTA-7199 and PTA-7201, respectively.

Antibodies that specifically bind to C-terminal epitopes located in human β -amyloid peptide, for use in the present invention include, but are not limited to, 369.2B, as described in U.S. Patent No. 5,786,180, entitled "Monoclonal antibody 369.2B specific for β A4 peptide." Further description of antibodies for use in the present invention can be found in, for example, Bussiere et al., (Am. J. Pathol. 165(3):987-95 (2004)) Bard et al. (PNAS 100(4):2023-8 (2003)), Kajkowski et al. (J. Biol. Chem. 276(22):18748-56 (2001)), Games et al. (Ann. NY Acad. Sci. 920:274-84 (2000)), Bard et al. (Nat. Med. 6(8):916-9 (2000)), and in International Patent Application No. WO03015691A2 entitled "Effecting rapid improvement of cognition in a subject having Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, or mild cognitive impairment, comprises administering anti-A beta antibody". Further description of antibody fragments for use in the present invention can be found in, for example, Bales et al. (Abstract P4-396, page S587, presented at Poster Session P4: Therapeutics and Therapeutic Strategies-Therapeutic Strategies, Amyloid-Based) and Zameer et al. (Abstract P4-420, page S593, presented at Poster Session P4: Therapeutics and Therapeutic Strategies-Therapeutic Strategies, Amyloid-Based).

Antibodies for use in the present invention may be recombinantly or synthetically produced. For example, the antibody may be produced by a recombinant cell culture process, using, for example, CHO cells, NIH 3T3 cells, PER.C6® cells, NS0 cells, VERO cells, chick embryo fibroblasts, or BHK cells. In addition, antibodies with minor

modifications that retain the primary functional property of binding A β peptide are contemplated by the present invention. In a particular embodiment, the antibody is a humanized anti A β peptide 3D6 antibody that selectively binds A β peptide. More specifically, the humanized anti A β peptide 3D6 antibody is designed to specifically bind
5 to an NH₂-terminal epitope, for example, amino acid residues 1-5, located in the human β -amyloid 1-40 or 1-42 peptide found in plaque deposits in the brain (for example, in patients suffering from Alzheimer's disease).

Figure 1 provides a schematic representation of the predicted structure of an exemplary humanized anti A β peptide antibody. The complete amino acid sequences of
10 the h3D6v2 light and heavy chains predicted from the DNA sequences of the corresponding expression vectors are shown in Figure 2 (where the residues are numbered starting with the NH₂-terminus of light and heavy chains as residue number 1) and in SEQ ID NO: 1 and SEQ ID NO:2, respectively. The last amino acid residue encoded by the heavy chain DNA sequence, Lys⁴⁴⁹, has not been observed in the mature,
15 secreted form of h3D6v2 and, without wishing to be bound to any particular theory, is presumably removed during intracellular processing by CHO cellular proteases. Therefore, the COOH-terminus of the h3D6v2 heavy chain is optionally Gly⁴⁴⁸. COOH-terminal lysine processing has been observed in recombinant and plasma-derived antibodies and does not appear to impact their function (Harris (1995) *J. Chromatogr. A.*
20 705:129-134). Purified h3D6v2 is post-translationally modified by addition of N-linked glycans to the Fc portion of heavy chain, which is known to contain a single N-glycosylation consensus site. The N-glycosylation site displays three major complex biantennary neutral oligosaccharide structures commonly observed at the analogous N-glycosylation site of mammalian IgG proteins.

25 Another exemplary humanized anti A β peptide antibody is humanized 3D6 version 1 (hu3D6v1) having the sequence set forth in Figure 2 but for a D \rightarrow Y substitution at position 1 of the light chain.

In various embodiments of the present invention, the anti A β antibody (for example, a humanized anti A β peptide 3D6 antibody) is present from about 0.1 mg/ml to
30 about 100 mg/ml, from about 0.1 mg/ml to about 75 mg/ml, from about 0.1 mg/ml to about 50 mg/ml, from about 0.1 mg/ml to about 40 mg/ml, from about 0.1 mg/ml to about 30 mg/ml, from about 10 mg/ml to about 20 mg/ml, from about 20 mg/ml to 30 mg/ml, or higher, for example, up to about 100 mg/ml, about 200 mg/ml, about 500

mg/ml, or about 1000 mg/ml or more. Preferably the anti A β antibody is present in a concentration of about 17 mg/ml to about 23 mg/ml. In various embodiments, the anti A β antibody is present at about 1, 2, 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 30 mg/ml. In a particular embodiment, the antibody (for example, a humanized anti A β peptide 3D6 antibody) is present at about 17 mg/ml. In another particular embodiment, the antibody (for example, a humanized anti A β peptide 3D6 antibody) is present at about 20 mg/ml. In another particular embodiment, the antibody (for example, a humanized anti A β peptide 3D6 antibody) at about 30 mg/ml. Ranges intermediate to the above recited concentrations, for example, about 12 mg/ml to about 17 mg/ml, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

Excipients

In various embodiments, the present invention provides a formulation that may include various excipients, including, but not limited to, buffer, anti-oxidant, a tonicity agent, and a stabilizer. In addition, the formulations may contain an additional agent for pH adjustment (for example, HCl) and a diluent (for example, water). In other embodiment, different forms of histidine can be used for pH adjustment. In part, the excipients serve to maintain the stability and the biological activity of the antibody (for example, by maintaining the proper conformation of the protein), and/or to maintain pH.

Buffering Agent

In various aspects of the present invention, the formulation includes a buffering agent (buffer). The buffer serves to maintain a physiologically suitable pH. In addition, the buffer can serve to enhance isotonicity and chemical stability of the formulation. Generally, the formulation should have a physiologically suitable pH. In various embodiments of the present invention, the formulation has a pH of about 5 to about 7, about 5.5 to about 6.5, preferably about 6.0 to about 6.5. In a particular embodiment, the formulation has a pH of about 6. Ranges intermediate to the above recited pH levels, for example, about pH 5.2 to about pH 6.3, preferably 6.0 or pH 6.2), are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be

included. The pH may be adjusted as necessary by techniques known in the art. For example, HCl may be added as necessary to adjust the pH to desired levels or different forms of histidine may be used to adjust the pH to desired levels.

The buffer may include, but is not limited to, succinate (sodium or phosphate),
5 histidine, phosphate (sodium or potassium), Tris (tris (hydroxymethyl) aminomethane), diethanolamine, citrate, other organic acids and mixtures thereof. In a preferred embodiment, the buffer is histidine (for example, L-histidine). In another particular embodiment, the buffer is succinate. In another embodiment, the formulation includes an amino acid such as histidine that is present in an amount sufficient to maintain the
10 formulation at a physiologically suitable pH. Histidine is an exemplary amino acid having buffering capabilities in the physiological pH range. Histidine derives its buffering capabilities spanning from its imidazole group. In one exemplary embodiment, the buffer is L-histidine (base) (for example $C_6H_9N_3O_2$, FW: 155.15). In another embodiment, the buffer is L-histidine monochloride monohydrate (for example
15 $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$, FW: 209.63). In another exemplary embodiment, the buffer is a mixture of L-histidine (base) and L-histidine monochloride monohydrate.

In one embodiment, the buffer (for example, L-histidine or succinate) concentration is present from about 0.1 mM to about 50 mM, from about 0.1 mM to about 40 mM, from about 0.1 mM to about 30 mM, about 0.1 mM to about 25 mM,
20 from about 0.1 mM to about 20 mM, or from about 5 mM to about 15 mM, preferably 5 mM or 10 mM. In various embodiments, the buffer may be present at about 6 mM, 7 mM, 8 mM, 9 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15 mM. In a particular embodiment, the buffer is present at about 10 mM. Ranges intermediate to the above recited concentrations, for example, about 12 mM to about 17 mM, are also intended to
25 be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In certain embodiments, the buffer is present in an amount sufficient to maintain a physiologically suitable pH.

30 Tonicity Agent

In various aspects of the present invention, the formulation includes a tonicity agent. In part, the tonicity agent contributes to maintaining the isotonicity of the formulation, and to maintaining protein levels. In part, the tonicity agent contributes to

preserving the level, ratio, or proportion of the therapeutically active polypeptide present in the formulation. As used herein, the term "tonicity" refers to the behavior of biologic components in a fluid environment or solution. Isotonic solutions possess the same osmotic pressure as blood plasma, and so can be intravenously infused into a subject without changing the osmotic pressure of the subject's blood plasma. Indeed, in one embodiment according to the invention, tonicity agent is present in an amount sufficient to render the formulation suitable for intravenous infusion. Often, the tonicity agent serves as a bulking agent as well. As such, the agent may allow the protein to overcome various stresses such as freezing and shear.

The tonicity agent may include, but is not limited to, CaCl_2 , NaCl , MgCl_2 , lactose, sorbitol, sucrose, mannitol, trehalose, raffinose, polyethylene glycol, hydroxyethyl starch, glycine and mixtures thereof. In a preferred embodiment, the tonicity agent is mannitol (for example, D-mannitol, for example, $\text{C}_6\text{H}_{14}\text{O}_6$, FW: 182.17).

In one embodiment, the tonicity agent is present at about 2% to about 6% w/v, or about 3% to about 5% w/v. In another embodiment, the tonicity agent is present at about 3.5% to about 4.5% w/v. In another embodiment, the tonicity agent is present at about 20 mg/ml to about 60 mg/ml, at about 30 mg/ml to about 50 mg/ml, or at about 35 mg/ml to about 45 mg/ml. Preferably, the tonicity agent is present at about 4% w/v or at about 40 mg/ml. In another particular embodiment, the tonicity agent is present at about 6% w/v. In yet another particular embodiment, the tonicity agent is present at about 10% w/v.

Ranges intermediate to the above recited concentrations, for example, about 3.2% to about 4.3% w/v or about 32 to about 43 mg/ml, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. The tonicity agent should be present in a sufficient amount so as to maintain tonicity of the formulation.

Anti-Oxidant

In various aspects of the present invention, the formulation includes an anti-oxidant so as to, in part, preserve the formulation (for example, by preventing oxidation).

The anti-oxidant may include, but is not limited to, GLA (gamma-linolenic acid)-lipoic acid, DHA (docosahexaenoic acid)-lipoic acid, GLA-tocopherol, di-GLA-3,3'-thiodipropionic acid and in general any of, for example, GLA, DGLA (dihomo-gamma-linolenic acid), AA (arachidonic acid), SA (salicylic acid), EPA
5 (eicosapentaenoic acid) or DHA (docosahexaenoic acid) with any natural or synthetic anti-oxidant with which they can be chemically linked. These include phenolic anti-oxidants (for example, eugenol, carnosic acid, caffeic acid, BHT (butylated hydroxyanisol), gallic acid, tocopherols, tocotrienols and flavenoid anti-oxidants (such as myricetin and fisetin)), polyenes (for example, retinoic acid), unsaturated sterols (for
10 example, Δ^5 -avenosterol), organosulfur compounds (for example, allicin), terpenes (for example, geraniol, abietic acid) and amino acid antioxidants (for example, methionine, cysteine, carnosine). In one embodiment, the anti-oxidant is ascorbic acid. Preferably, the anti-oxidant is methionine, or an analog thereof, for example, selenomethionine, hydroxy methyl butanoic acid, ethionine, or trifluoromethionine.

15 In one embodiment, the anti-oxidant (for example, a methionine such as L-methionine, for example $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$, FW=149.21) is present from about 0.1 mM to about 50 mM, from about 0.1 mM to about 40 mM, from about 0.1 mM to about 30 mM, from about 0.1 mM to about 20 mM, or from about 5 mM to about 15 mM. In various embodiments, the anti-oxidant may be present at about 5 mM,
20 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15 mM. Preferably, the anti-oxidant is present at about 10 mM. In another particular embodiment, the anti-oxidant is present at about 15 mM. Ranges intermediate to the above recited concentrations, for example, about 12 mM to about 17 mM, are also intended to be part of this invention. For example, ranges of values using a combination
25 of any of the above recited values as upper and/or lower limits are intended to be included. In certain embodiments, the anti-oxidant should be present in a sufficient amount so as to preserve the formulation, in part, by preventing oxidation.

Stabilizer

30 In various aspects of the present invention, the formulation includes a stabilizer, also known as a surfactant. Stabilizers are specific chemical compounds that interact and stabilize biological molecules and/or general pharmaceutical excipients in a formulation. In certain embodiments, stabilizers may be used in conjunction with lower

temperature storage. Stabilizers generally protect the protein from air/solution interface induced stresses and solution/surface induced stresses, which may otherwise result in protein aggregation.

The stabilizer may include, but is not limited to, glycerin, polysorbates such as polysorbate 80, dicarboxylic acids, oxalic acid, succinic acid, adipic acid, fumaric acid, phthalic acids, and combinations thereof. In a preferred embodiment the stabilizer is polysorbate 80.

In one embodiment, the stabilizer (for example, polysorbate 80) concentration is about 0.001% w/v to about 0.01% w/v, about 0.001% w/v to about 0.009% w/v, or about 0.003% w/v to about 0.007% w/v. Preferably, the stabilizer concentration is about 0.005% w/v. In another particular embodiment, the stabilizer is present at about 0.01% w/v. Ranges intermediate to the above recited concentrations, for example, about 0.002% w/v to about 0.006% w/v, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. The stabilizer should be present in a sufficient amount so as to stabilize the A β binding polypeptide (for example, anti A β antibody).

Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. In a particular embodiment, the formulation is substantially free of preservatives, although, in alternative embodiments, preservatives may be added as necessary. For example, cryoprotectants or lyoprotectants may be included, for example, should the formulation be lyophilized.

In various aspects of the present invention, the formulations optionally include some or all of the classes of excipients described above. In one aspect, the formulations of the present invention include A β binding polypeptide (for example, anti A β antibody), mannitol and histidine. In particular embodiments, the formulations may include an anti-oxidant such as methionine, and/or a stabilizer such as polysorbate 80. In certain embodiments, the formulations have a pH of about 6. In another aspect, the formulation includes an A β binding polypeptide (for example, an anti A β antibody), mannitol, histidine and methionine. In yet another aspect, the formulation includes an A β binding polypeptide (for example, an anti A β antibody), mannitol, histidine,

methionine and polysorbate 80. In a particular aspect of the invention, the formulation includes about 20 mg/ml an A β binding polypeptide (for example, an anti A β antibody), about 10 mM histidine, about 10 mM methionine, about 4% mannitol and has a pH of about 6. In another aspect of the invention, the formulation includes about 20 mg/ml A β binding polypeptide (for example, anti A β antibody), 10 mM histidine, 10 mM methionine, 4% w/v mannitol, 0.005% w/v polysorbate 80 and has a pH of about 6. A preferred formulation includes about 17 mg/ml to about 23 mg/ml of a humanized 3D6 antibody, about 10 mM histidine, about 10 mM methionine, about 4% w/v mannitol, about 0.005% polysorbate 80 and has a pH of about 5.5 to about 6.5. Another preferred formulation includes about 10 mg/ml to about 30 mg/ml of a humanized 266 antibody, about 10 mM histidine or succinate, about 10 mM methionine, about 4% w/v mannitol or sorbitol and has a pH of about 5.5 to about 6.5. Yet another preferred formulation includes about 10 mg/ml to about 30 mg/ml of a humanized 12A11 antibody, about 5 mM histidine, about 10 mM methionine, about 4% mannitol or 150 mM NaCl, and has a pH of about 5.5 to about 6.5. Another formulation is stable for at least about 12 months at a temperature of above freezing to about 10°C, has a pH of about 5.5 to about 6.5, and includes at least one anti A β antibody at a concentration of about 1 mg/ml to about 30 mg/ml, mannitol at a concentration of about 4% w/v or NaCl at a concentration of about 150 mM, about 5 mM to about 10 mM histidine or succinate, and 10 mM methionine. Preferably, the formulation also includes polysorbate at a concentration of about 0.001% w/v to about 0.01% w/v.

Exemplary embodiments of the present invention provide concentrated preparations of A β binding polypeptide (for example, anti A β antibody), often useful as bulk drug product. Furthermore, exemplary embodiments of the present invention are stable to freezing, lyophilization and/or reconstitution. Moreover, exemplary embodiments of the present invention are stable over extended periods of time. For example, the formulations of the present invention are stable for at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 months. In particular embodiments, the formulations of the present invention are stable for at least about 12 months, for at least about 18 months, for at least about 24 months, or for at least about 30 months.

According to the invention, the formulation may be stored at temperatures from about -80°C to about 40°C, from about 0°C to about 25°C, from about 0°C to about 15°C,

or from about 0°C to about 10°C, preferably from about 2°C to about 8°C. In various embodiments, the formulation may be stored at about 0°C, 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C or 10°C. In a particular embodiment, the formulation is stored at about 5°C. Generally, the formulation is stable and retains biological activity at these ranges.

- 5 Ranges intermediate to the above recited temperatures, for example, from about 2°C to about 17°C, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

10 The formulations of the present invention are suitable for delivery by a variety of techniques. In certain embodiments, the formulation is administered parenterally, such as intravenously or intramuscularly. Additionally, one may target delivery of the formulation to the brain (for example, so that the antibody may cross the blood brain barrier) or the spinal fluid. In a particular embodiment, the formulation is administered intravenously.

15 Effective doses of the formulations of the present invention vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated.

20 Treatment dosages need to be titrated to optimize safety and efficacy.

For passive immunization with an antibody, exemplary dosages are from about 0.0001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 5 mg/kg, about 0.15 mg/kg to about 3 mg/kg, 0.5 mg/kg to about 2 mg/kg, preferably about 1 mg/kg to about 2 mg/kg of the host body weight. In some exemplary embodiments, dosages can be about 25 0.5, 0.6, 0.7, 0.75, 0.8, 0.9, 1.0, 1.2, 1.25, 1.3, 1.4, 1.5, 1.6, 1.7, 1.75, 1.8, 1.9, or 2.0 mg/kg. Other exemplary dosages for passive immunization are from about 1 mg/kg to about 20 mg/kg. In some exemplary embodiments, dosages can be about 5, 10, 15 or 20 mg/kg. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary 30 treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30

mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

5 Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to A β in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μ g/ml and in some methods 25-300 μ g/ml. Alternatively, antibody can be administered as a sustained
10 release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies.

 The dosage and frequency of administration can vary depending on whether the
15 treatment is prophylactic or therapeutic. In prophylactic applications, formulations containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from
20 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives.

 In some therapeutic applications, a relatively high dosage (for example, from about 0.5 or 1 to about 200 mg/kg of antibody per dose (for example 0.5, 1, 1.5, 2, 5, 10,
25 20, 25, 50, or 100 mg/kg), with dosages of from 5 to 25 mg/kg being more commonly used) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

30 It is especially advantageous to provide the formulations of the invention in unit dosage form for ease of administration and uniformity of dosage. Formulations of the invention may be presented in capsules, ampules, lyophilized form, or in multi-dose

containers. The term "container" refers to something, for example, a holder, receptacle, or vessel, into which an object or liquid can be placed or contained, for example, for storage. The unit dosage form may comprise any formulation described herein including suspensions, solutions or emulsions of the active ingredient together with formulating agents such as suspending, stabilizing and/or dispersing agents. In an exemplary embodiment, the pharmaceutical dosage unit form may be added to an intravenous drip bag (for example a 50 ml, 100 ml, or 250 ml, or 500 ml drip bag) with a suitable diluent, for example, sterile pyrogen-free water or saline solution, before administration to the patient, for example, by intravenous infusion. Some pharmaceutical unit dosage forms may require reconstitution with a suitable diluent prior to addition to an intravenous drip bag, particularly lyophilized forms. In exemplary embodiments, the pharmaceutical unit dosage form is a container containing a formulation described herein. For example, the container may be a 10 mL glass, type I, tubing vial. Generally, the container should maintain the sterility and stability of the formulation. For example, the vial may be closed with a serum stopper. Furthermore, in various embodiments, the container should be designed so as to allow for withdrawal of about 100 mg of formulation or active ingredient (for example, for single use). Alternatively, the container may be suitable for larger amounts of formulation or active ingredient, for example, from about 10 mg to about 5000 mg, from about 100 mg to about 1000 mg, and from about 100 mg to about 500 mg, about 40 mg to about 250 mg, about 60 mg to about 80 mg, about 80 mg to about 120 mg, about 120 mg to about 160 mg, or ranges or intervals thereof, for example, about 100 mg to about 200 mg. Ranges intermediate to the above recited amounts, for example, from about 25 mg to about 195 mg, are also intended to be part of this invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In a particular embodiment, the formulation often is supplied as a liquid in unit dosage form.

In another aspect, the present invention provides a kit including a pharmaceutical dosage unit form (for example, a container with a formulation disclosed herein), and instructions for use. Accordingly, the container and the kit may be designed to provide enough formulation for multiple uses. In various embodiments, the kit may further include diluent. The diluent may include excipients, separate or combined. For example, the diluent may include a tonicity modifier such as mannitol, a buffering agent such as histidine, a stabilizer such as polysorbate 80, an anti-oxidant such as methionine,

and/or combinations thereof. The diluent may contain other excipients, for example, lyoprotectant, as deemed necessary by one skilled in the art.

Additional useful embodiments of the invention are set forth in the section of this application entitled "Summary of the Invention".

5 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the figures, are incorporated herein by reference.

10 EXAMPLES

In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, recombinant DNA technology, immunology (especially, for example, antibody technology), and standard techniques of polypeptide preparation. See, for example, Sambrook, Fritsch and
15 Maniatis, *Molecular Cloning*: Cold Spring Harbor Laboratory Press (1989); *Antibody Engineering Protocols* (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); *Antibody Engineering: A Practical Approach* (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); *Antibodies: A Laboratory Manual*, Harlow *et al.*, C.S.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel
20 *et al.*, John Wiley & Sons (1992).

Example I. Cloning and Expression of Humanized Anti A Beta Antibody

An exemplary antibody for formulation according to the methods of the instant invention is 3D6. The 3D6 mAb is specific for the N-terminus of A β and has been
25 shown to mediate phagocytosis (for example, induce phagocytosis) of amyloid plaque. 3D6 does not recognize secreted APP or full-length APP, but detects only A β species with an amino-terminal aspartic acid. Therefore, 3D6 is an end-specific antibody. The cell line designated RB96 3D6.32.2.4 producing the antibody 3D6 has the ATCC accession number PTA-5130, having been deposited on Apr. 8, 2003. The cloning,
30 characterization and humanization of 3D6 antibody is described in U.S. Patent Application Publication No. 20030165496 A1. Briefly, humanization of the anti A β peptide murine monoclonal antibody (designated as m3D6) was carried out by isolating the DNA sequences for m3D6 light chain and heavy chain variable regions (V_L and V_H)

by reverse transcription - polymerase chain reaction (RT-PCR). Based on the determined m3D6 V_L and V_H DNA sequences, homologous human framework regions were identified. To insure that the humanized antibody retained the ability to interact with the A β peptide antigen, critical murine V_L and V_H framework residues were retained in the humanized 3D6 sequence to preserve the overall structure of the constant domain regions (CDRs) in the context of human kappa light chain and IgG1 heavy chain sequences. DNA sequences encoding the humanized 3D6 V_L and V_H sequences identified by this process (including the 5' signal peptide sequence and 3' intron splice-donor sequence) were generated by annealing synthesized overlapping DNA oligonucleotides followed by DNA polymerase fill-in reactions. The integrity of each of the humanized variable region sequences was verified by DNA sequencing. *Figure 1* depicts a schematic representation of the predicted structure of an exemplary humanized anti A β peptide 3D6 antibody termed h3D6v2. *Figure 2* identifies the complete amino acid sequences of the h3D6v2 light and heavy chains.

Humanized 3D6 antibody was expressed by transfection of a Chinese Hamster Ovary (CHO) host cell lineage with expression plasmids encoding anti A β antibody light chain and heavy chain genes. CHO cells expressing the antibody were isolated using standard methotrexate - based drug selection/gene amplification procedures. A clonal CHO cell line exhibiting the desired productivity and growth phenotypes was selected and used to establish an antibody expressing cell line using chemically defined medium free of animal or human - derived components.

Example II. Manufacturing Humanized Anti A β Antibody drug substance

The polypeptide manufacturing process began with the thawing of a starter culture of clonal cells stably expressing the anti-A β antibody. Cells were cultured using a chemically defined medium containing no animal or human-derived proteins. Cultures were then expanded and used to inoculate a seed bioreactor, which in turn was used to inoculate multiple production bioreactor cycles. The production bioreactor was operated in fed-batch mode. At the end of the production cycle, the conditioned medium harvest was clarified by microfiltration in preparation for further downstream processing.

The purification processes consisted of standard chromatographic steps followed by filtration. Purified antibody was concentrated by ultrafiltration and diafiltered into formulation buffer absent polysorbate-80. Optionally, polysorbate 80 (vegetable

derived) is added to the ultrafiltration/diafiltration retentate pool, followed by bacterial retention filtration. The drug substance was stored frozen at -80°C and held for further manufacture into drug product, including stabilized liquid formulations described herein.

5 **Example III. Preparation of Antibody Formulation and Placebo**

Two batches of antibody drug product were manufactured. An initial batch was manufactured by compounding drug substance into an animal and human protein-free formulation containing 20 mg anti A β antibody active substance per mL, 10 mM histidine, 10 mM methionine, 4% mannitol, 0.005% polysorbate-80, pH 6.0. The drug
10 product was aseptically filled into vials, at 100 mg anti A β antibody active substance/vial. The finished drug product vial contained no preservative and was intended for single-use only.

A second batch of drug product was manufactured by a similar method using a formulation buffer without polysorbate-80.

15

Example IV. Stability Analysis of Formulations with and without Polysorbate-80

The stability and, in particular, the physicochemical integrity (such as aggregation, deamidation, hydrolysis, and/or disulfide bond rearrangement) of the
20 formulation were assessed by the following methods well known in the art: appearance; pH; protein concentration (A280); ELISA, in part, as a test of bioactivity; sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), in part as a test of aggregation; size exclusion high performance liquid chromatography (SEC-HPLC), in part, as a test of aggregation and stability in general; cation exchange high performance
25 liquid chromatography (CEX-HPLC), in part, as a test of deamidation and stability in general; and peptide mapping. These methods assessed the recovery and integrity of the protein under test conditions at various temperatures.

Appearance analysis of the formulations was conducted in order to determine the quality of the formulations at various time points. Analysis was conducted based on
30 visual inspection for clarity, color and the presence of particulates. For example, the degree of opalescence was analyzed in terms of reference suspensions. Appearance analysis of the formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were acceptable when stored

at each of -80°C, 5°C, 25°C, and 40°C at each of the following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months.

A pH analysis was carried out to determine the maintenance of the formulation's pH within an acceptable range of about 5.5 to about 6.5. pH analysis of formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were acceptable when stored at each of -80°C, 5°C, 25°C, and 40°C at each of the following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months. Generally, the pH never ranged below 5.8 or above 6.2.

Protein concentration analysis by A280 assays was performed to determine the maintenance of the formulation's protein concentration within an acceptable range of about 17 mg/ml to about 23 mg/ml. Protein concentration analysis of formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were generally acceptable when stored at each of -80°C, 5°C, 25°C, and 40°C at each of the following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months. With the exception of the protein concentrations ranging slightly above 23 mg/ml for the formulation without polysorbate 80 when stored at 5°C, 25°C, and 40°C at the 3 month timepoints, the protein concentration otherwise remained within the acceptable ranges. Accordingly, the protein concentration analysis demonstrated no detectable loss of protein occurring, even at accelerated conditions, particularly for the formulations with polysorbate 80. Moreover, protein concentration generally failed to demonstrate a significant time or temperature dependent change subsequent to the initial time point.

Maintenance of biological activity was assayed, in part, by ELISA techniques.

Biological activity was analysed as binding units (BU)/mg with acceptable activity being ≥ 2500 BU/mg or 50% (*i.e.*, 5000 BU/mg equates to 100%). ELISA analysis of formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were generally acceptable when stored at each of -80°C, 5°C, 25°C, and 40°C at each of the following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months. With the exception of the biological activity ranging slightly below 50% at the 12 month time point for both formulations when stored at 40°C, the biological activity otherwise remained within the acceptable ranges.

SEC-HPLC analysis was conducted as a test of aggregation, purity and stability in general. SEC-HPLC runs under conditions using mobile phase chromatography with a sodium phosphate dibasic buffer indicated the formulation was acceptable if the SEC-HPLC analysis identified $\geq 90\%$ IgG monomer, compared to percentage of high
5 molecular weight product and low molecular weight product. SEC-HPLC analysis of formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were generally acceptable when stored at each of -80°C , 5°C , 25°C , and 40°C at each of the following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months. With the exception of the
10 percentage monomer ranging below 90% for both formulations when stored at 40°C at each time point at and after 6 months (where the analysis identified greater than at least 10% low molecular weight product for both formulations at each time point), percentage monomer was otherwise within the acceptable range. SEC-HPLC analysis generally demonstrated that although the high molecular weight and low molecular weight profiles
15 were different over time in samples with and without polysorbate, the monomeric form of the antibody generally remained constant, for example at the 12 month time point, when the formulation was stored at 5°C .

CEX-HPLC analysis was conducted as a test of amination and stability in general. CEX-HPLC runs under conditions using mobile phase chromatography with a
20 NaCl buffer produced elution profile and retention times of predominant peaks which were analyzed as being comparable or not comparable to reference standard profiles. CEX-HPLC analysis of formulations made with and without polysorbate 80 in accordance with the present invention demonstrated that both formulations were generally acceptable when stored at each of -80°C , 5°C , 25°C , and 40°C at each of the
25 following timepoints: initial, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months. With the exception of the elution profile and retention time of the predominant peaks not being comparable for both formulations when stored at 40°C at each time point at and after 3 months, the predominant peaks were otherwise comparable to the reference peaks.

30 Generally, analysis of the formulations with polysorbate 80 stored at 5°C allow for the following particularly important conclusions: 1) opalescence, pH, ELISA, CEX-HPLC, SEC-HPLC and SDS PAGE analysis all showed minimal changes in the formulation over 9 months; 2) formulations stored at 5°C appeared more like reference

samples over 9 months than the accelerated samples; 3) peptide mapping showed changes at 5°C; and 4) SEC-HPLC trending data at 5°C predicted at least 17.2 months of stability (see Figure 6), however, upon removing column, instrument and buffer variability, the data allowed for a prediction of greater than 30 months of stability (see Figure 7). Additionally, accelerated samples with polysorbate 80 stored at 25°C passed all specifications at 9 months (Figure 4).

Moreover, analysis of the formulations without polysorbate 80 stored at 5°C allow for the following particularly important conclusions: 1) opalescence, pH and ELISA analysis all showed minimal changes in the formulation over 9 months; 2) results of the CEX-HPLC and SDS PAGE showed comparable findings to reference samples or the -80°C control at 9 months; 3) SEC-HPLC analysis showed minor changes over 9 months while changes were more pronounced at accelerated temperatures; and 4) SEC-HPLC trending data predicted at least 18 months of stability, even with assay variability issues (see Figure 8).

Figures 3-5 are graphical depictions of the shelf life predictions for the formulations (with and without PS80) made in accordance with the present invention and stored at 5°C, 25°C, and 40°C, respectively. Generally, Figures 3-5 indicate that storage of the formulations of the present invention at higher temperatures reduces the expected shelf life. Figure 3, in particular, indicates that the formulation has an expected shelf life of at least 18 months when the formulation is stored at 5°C. Figure 4 indicates that storage of the formulation at room temperature (25°C) may serve to reduce expected shelf life to about 12 months. Figure 5 further demonstrates that storage of the formulation at 40°C may serve to reduce expected shelf life to about 4 months.

Example V. Stability Studies on Use of Methionine as an Anti-Oxidant

Studies were conducted to determine the effect of methionine on maintaining the stability of the antibody in antibody formulations. SEC-HPLC analysis was conducted over 6 months at various temperatures on four antibody samples (using an anti-CD22 IgG₄ antibody): an antibody formulation with 20 mM succinate at a pH of 6.0; an antibody formulation with 20 mM succinate and 10 mM methionine; an antibody formulation with 20 mM succinate and 0.01% PS80; and an antibody formulation with 20 mM succinate, 10 mM methionine and 0.01% PS80. Generally, the results indicated that methionine desirably lessens high molecular weight (HMW) formation. Moreover,

methionine decreases temperature dependent increase in the percent of HMW (See Figure 10).

Furthermore, a pH stability study (at pH 5.8, 6.0 and 6.2) was conducted over 6 weeks at various temperatures (5°C and 40°C) on the following four antibody (an anti-B7.2 IgG₂ antibody) samples: (1) a sample including antibody, 10 mM histidine and 150 mM NaCl; (2) a sample including antibody, 10 mM histidine, 150 mM NaCl and 0.01% PS80; (3) a sample including antibody, 10 mM histidine, 150 mM NaCl and 10 mM methionine; and (4) a sample including antibody, 10 mM histidine, 150 mM NaCl, 10 mM methionine and 0.01% PS80. SEC-HPLC analysis was conducted. The results demonstrated that methionine decreases the temperature dependent increase in percent of by-product formation (for example, HMW by-products) over the indicated pH range, for example, from about pH 5.8 to about pH 6.2 (see Figure 11). As shown in Figure 11, samples containing methionine displayed a low amount of aggregation when maintained at 40°C for six weeks, which was similar to that for samples maintained at 5°C for six weeks.

Example VI. Excipient Analysis of an IgG1 Antibody by Differential Scanning Calorimetry

A primary goal of protein drug formulation is to stabilize a protein in its native, biologically active form. Typically this can be done by screening various excipients in a base formulation and monitoring their effect on the molecule's molecular weight and activity. These parameters are indicative of stability. Another measurement of stability is thermal denaturation which can be monitored using a variety of biophysical techniques. Generally, increased levels of protein stability have been attributed to high melting, denaturation or decomposition temperatures. Accordingly, thermal properties of a representative IgG1 monoclonal antibody were monitored in the presence of various excipients using a VP-Capillary Differential Scanning Calorimeter. Specifically, the apparent T_m s were determined for formulations containing 10 mM histidine (pH 6.0) with various excipients. Several excipients were shown to provide increased or decreased thermal stability. Because increased levels of protein stability have been attributed to a high melting temperature, excipients in samples imparting an increased T_{m2} or T_{m3} , as compared to control T_{m2} / T_{m3} values (respectively, 74.9°C and 83.4°C), were deemed to be especially desirable excipients (see Table 1 below).

Accordingly, it was concluded that excipients such as glucose (formulated at a concentration of 4% and 10%), sucrose (formulated at a concentration of 4% and 10%), sorbitol (formulated at a concentration of 4% and 10%), and mannitol (formulated at a concentration of 4% and 10%), performed especially well in stabilizing a liquid polypeptide formulation, in particular, an antibody IgG formulation.

Table 1 Excipient Analysis Results

Excipient	Concentration	T _m 1*	T _m 2*	T _m 3*
Histidine (Control)	10 mM	-	74.9	83.4
NaCl	10 mM	69.3	74.8	82.9
	100 mM	67.9	74.4	82.4
	500 mM	66.5	74.5	81.9
	1 M	65.4	74.9	82.3
CaCl ₂	10 mM	68.7	74.6	82.7
	100 mM	68.5	74.5	82.4
Methionine	30 mM	-	74.5	83.7
Vitamin C	~30 mM	52.2	68.7	-
Polysorbate 20	0.005%	-	74.5	83.7
	0.01%	-	74.5	83.8
	0.1%	-	74.4	83.7
Polysorbate 80	0.005%	-	74.6	83.8
	0.01%	-	74.5	83.7
	0.1%	-	74.5	83.7
Glucose	0.5%	-	74.7	83.8
	2%	-	74.9	83.9
	4%	-	75.0	84.3
	10%	-	75.8	84.9
Sucrose	0.5%	-	74.6	83.6
	2%	-	74.8	83.8
	4%	-	75.0	83.9
	10%	-	75.5	84.4
Sorbitol	0.5%	-	74.8	83.6
	2%	-	75.0	83.8
	4%	-	75.2	84.1
	10%	-	75.9	84.8
Mannitol	0.5%	-	74.8	83.6
	2%	-	74.9	83.8
	4%	-	75.2	84.1
	10%	-	75.9	84.8

*In the control (10 mM histidine, pH 6.0) two transitions were observed, T_m2 and T_m3. An earlier transition (T_m1) was seen in the presence of some excipients.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.

We claim:

1. A stabilized formulation comprising at least one A β binding polypeptide, at least one tonicity agent, wherein the tonicity agent is present in an amount sufficient to render the formulation suitable for administration, and at least one buffering agent in an amount sufficient to maintain a physiologically suitable pH.
2. The formulation of claim 1, further comprising at least one antioxidant.
3. The formulation of claim 2, wherein at least one antioxidant is methionine.
4. The formulation of claim 1, wherein at least one tonicity agent is mannitol.
5. The formulation of claim 4, wherein at least one tonicity agent is NaCl.
6. The formulation of claim 1, wherein at least one buffering agent is histidine.
7. The formulation of claim 1, wherein at least one buffering agent is succinate.
8. The formulation of claim 1, wherein at least one buffering agent is sodium phosphate.
9. The formulation of claim 1, further comprising at least one stabilizer.
10. The formulation of claim 9, wherein at least one stabilizer is polysorbate 80.
11. The formulation of claim 1, wherein at least one A β binding polypeptide is selected from the group consisting of an anti A β antibody, an anti A β antibody Fv fragment, an anti A β antibody Fab fragment, an anti A β antibody Fab'(2) fragment, an anti A β antibody Fd fragment, a single-chain anti A β antibody (scFv), a single domain anti A β antibody fragment (Dab), a beta-pleated sheet polypeptide comprising at least one antibody complementarity determining

region (CDR) from an anti A β antibody, and a non-globular polypeptide comprising at least one antibody CDR from an anti A β antibody.

5 12. The formulation of claim 11, wherein at least one A β binding polypeptide is an anti A β antibody.

13. The formulation of claim 12, wherein the anti A β antibody specifically binds to an epitope within residues selected from the group consisting of 1-7, 1-5, 3-7, 3-6, 13-28, 15-24, 16-24, 16-21, 19-22, 33-40, and 33-42 of A β .

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14. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 1-7.

15 15. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 1-5.

16. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 3-6.

20 17. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 3-7.

18. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 13-28.

25

19. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 16-24.

30 20. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 16-21.

21. The formulation of claim 13, wherein the anti A β antibody specifically binds to an epitope within residues 19-22.
22. The formulation of claim 12, wherein the anti A β antibody is selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, and a humanized 15C11 antibody.
23. The formulation of claim 22, further comprising a stabilizer and an antioxidant.
24. The formulation of claim 23, wherein the stabilizer is polysorbate 80, the antioxidant is methionine, the tonicity agent is mannitol, sorbitol or NaCl, and the buffering agent is histidine.
25. The formulation of claim 22, wherein the formulation is a lyophilized formulation.
26. The formulation of claim 22, wherein the formulation is a liquid formulation.
27. The formulation of claim 22, wherein the antibody is of isotype human IgG1.
28. The formulation of claim 22, wherein the antibody is of isotype human IgG4.
29. The formulation of claim 12, wherein the anti A β antibody is present at a concentration of about 0.1 mg/ml to about 60 mg/ml.
30. The formulation of claim 12, wherein the anti A β antibody is present at a concentration of about 20 mg/ml.
31. The formulation of claim 12, wherein the anti A β antibody is present at a concentration of about 10 mg/ml.

32. The formulation of claim 23, wherein at least one tonicity agent is D-mannitol at a concentration of about 1% w/v to about 10% w/v.
- 5 33. The formulation of claim 32, wherein the concentration of D-mannitol is about 2% w/v to about 6% w/v.
34. The formulation of claim 23, wherein at least one buffering agent is histidine at a concentration of about 0.1 mM to about 25 mM.
- 10 35. The formulation of claim 23, wherein the formulation has a pH of about 5.5 to about 6.5.
36. The formulation of claim 22, wherein the anti A β antibody is a humanized 3D6 antibody.
- 15 37. The formulation of claim 22, wherein the anti A β antibody is a humanized 12A11 antibody.
38. The formulation of claim 22, wherein the anti A β antibody is a humanized 266 antibody.
- 20 39. The formulation of claim 22, wherein the anti A β antibody is a humanized 15C11 antibody.
- 25 40. A formulation stable for at least about 12 months at a temperature of above freezing to about 10°C and having a pH of about 5.5 to about 6.5, comprising:
- i. at least one A β antibody at a concentration of about 1 mg/ml to about 30 mg/ml;
 - ii. mannitol at a concentration of about 4% w/v or NaCl at a concentration of about 150 mM;
 - 30 iii. about 5 mM to about 10 mM histidine or succinate; and
 - iv. 10 mM methionine.

41. The formulation of claim 40, wherein the formulation is stable for at least about 24 months at a temperature of about 2°C to 8°C, and comprises polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v.

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42. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to about 6.5 and comprises about 10 mg/ml A β antibody, about 10 mM histidine and about 4% w/v mannitol and about .005% w/v polysorbate 80.

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43. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to about 6.2 and comprises about 20 mg/ml A β antibody, about 10 mM histidine, about 4% w/v mannitol and about .005% w/v polysorbate 80.

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44. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to about 6.2 and comprises about 30 mg/ml A β antibody, about 10 mM histidine, about 4% w/v mannitol and about .005% w/v polysorbate 80.

45. The formulation of claim 40, wherein the A β antibody is selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, a humanized 266 antibody, a humanized 12A11 antibody, and a humanized 15C11 antibody.

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46. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to 6.5 and comprises about 10 mM histidine, about 4% w/v mannitol and about 2 mg/ml to about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 3D6 antibody, a humanized 10D5 antibody, a humanized 12B4 antibody, and a humanized 12A11 antibody.

25

47. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to 6.5 and comprises about 10 mM histidine, about 150 mM NaCl and about 2 mg/ml to

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about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 12B4 antibody, and a humanized 12A11 antibody.

5 48. The formulation of claim 40, wherein the formulation has a pH of about 6.0 to 6.5 and comprises about 10 mM histidine, about 4% w/v mannitol and about 2 mg/ml to about 20 mg/ml of an A β antibody selected from the group consisting of a humanized 266 antibody and a humanized 15C11 antibody.

10 49. A formulation stable for at least about 24 months at a temperature of about 2°C to about 8°C and having a pH of about 5.5 to about 6.5, comprising about 2 mg/ml to about 23 mg/ml of a humanized 3D6 antibody, about 10 mM histidine and about 10 mM methionine.

15 50. The formulation of claim 49, further comprising about 4% w/v mannitol.

51. The formulation of claim 49, further comprising polysorbate 80 at a concentration of about 0.001% w/v to about 0.01% w/v.

20 52. The formulation of claim 51, comprising about 0.005% w/v polysorbate 80.

53. The formulation of claim 49, wherein the humanized 3D6 antibody is present at a concentration of about 17 mg/ml to about 23 mg/ml.

25 54. A formulation stable for at least about 24 months at a temperature of about 2°C to about 8°C and having a pH of about 5.5 to about 6.5, comprising about 2 mg/ml to about 23 mg/ml of a humanized 3D6 antibody, about 10 mM succinate, about 10 mM methionine, about 4% w/v mannitol and about 0.005% w/v polysorbate 80.

30 55. A formulation stable for at least about 24 months at a temperature of about 2°C to about 8°C and having a pH of about 6.0 to about 6.5, comprising about 2 mg/ml to about 30 mg/ml of a humanized 266 antibody, about 10 mM histidine and about 10 mM methionine.

56. The formulation of claim 55, further comprising about 4% w/v mannitol.

57. The formulation of claim 55, further comprising polysorbate 80 at a
5 concentration of about 0.001% w/v to about 0.01% w/v.

58. A formulation stable for at least about 24 months at a temperature of about 2°C to
about 8°C and having a pH of about 6.0 to about 6.5, comprising about 2 mg/ml to
about 30 mg/ml of a humanized 12A11 antibody, about 10 mM histidine and about
10 10 mM methionine.

59. The formulation of claim 58, further comprising about 150 mM NaCl.

60. The formulation of claim 58, further comprising polysorbate 80 at a
15 concentration of about 0.001% w/v to about 0.01% w/v.

61. A formulation stable for at least about 24 months at a temperature of about 2°C to
about 8°C and having a pH of about 6.0 to about 6.5, comprising about 2 mg/ml to
about 20 mg/ml of a humanized 12A11 antibody, about 5 mM histidine, about 10
20 mM methionine, about 4% w/v mannitol and about 0.005% w/v polysorbate 80.

62. A formulation stable when thawed from about -50°C to about -80°C, comprising
about 40 to about 60 mg/ml of an anti A β antibody, about 1.0 mg/ml to about 2.0
mg/ml histidine, about 1.0mg/ml to 2.0 mg/ml methionine and about 0.05 mg/ml
25 polysorbate 80, wherein the formulation has a pH of about 6.0.

63. The formulation of claim 62, wherein mannitol is excluded.

64. The formulation of claim 63, wherein the A β antibody is a humanized 3D6
30 antibody.

65. The formulation of claim 63, wherein the A β antibody is a humanized 266 antibody.
- 5 66. A formulation comprising about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol and having a pH of about 6.
67. A formulation comprising about 30 mg/mL anti A β antibody, about 10 mM succinate, about 10 mM methionine, about 6% mannitol and having a pH of about 6.2.
- 10 68. A formulation comprising about 20 mg/mL anti A β antibody, about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol, about 0.005% polysorbate 80, and having a pH of about 6.
- 15 69. A formulation comprising about 10 mg/mL anti A β antibody, about 10 mM succinate, about 10 mM methionine, about 10% mannitol, about 0.005% polysorbate 80, and having a pH of about 6.5.
- 20 70. A formulation comprising about 5 mg/mL to about 20 mg/mL anti A β antibody, about 5 mM to about 10 mM L-histidine, about 10 mM methionine, about 4% mannitol, about 0.005% polysorbate 80, and having a pH of about 6.0 to about 6.5.
- 25 71. A formulation comprising about 5 mg/mL to about 20 mg/mL anti A β antibody, about 5 mM to about 10 mM L-histidine, about 10 mM methionine, about 150 mM NaCl, about 0.005% polysorbate 80, and having a pH of about 6.0 to about 6.5.
72. A pharmaceutical unit dosage form, comprising a formulation comprising:

- a. about 10 mg to about 250 mg of an anti A β antibody;
- b. about 4% mannitol or about 150 mM NaCl;
- c. about 5 mM to about 10 mM histidine or succinate; and
- d. about 10 mM methionine

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73. The pharmaceutical unit dosage form of claim 72, comprising about 0.001% to about 0.1% polysorbate 80.

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74. The pharmaceutical unit dosage form of claim 73, comprising about 40 mg to about 60 mg of the anti A β antibody.

75. The pharmaceutical unit dosage form of claim 73, comprising about 60 mg to about 80 mg of the anti A β antibody.

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76. The pharmaceutical unit dosage form of claim 73, comprising about 80 mg to about 120 mg of the anti A β antibody.

77. The pharmaceutical unit dosage form of claim 73, comprising about 120 mg to about 160 mg of the anti A β antibody.

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78. The pharmaceutical unit dosage form of claim 73, comprising about 160 mg to about 240 mg of the anti A β antibody

79. A therapeutic product, comprising:

25

- a. a glass vial, comprising a formulation comprising:
 - i. about 10 mg to about 250 mg of a humanized anti A β antibody,
 - ii. about 4% mannitol or about 150 mM NaCl,
 - iii. about 5 mM to about 10 mM histidine, and
 - iv. about 10 mM methionine; and

30

- b. labeling for use comprising instructions to use the appropriate volume necessary to achieve a dose of about 0.15 mg/kg to about 5 mg/kg.

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80. The therapeutic product of claim 79, wherein the dose is about .5 mg/kg to about 3 mg/kg.

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81. The therapeutic product of claim 79, wherein the dose is about 1 mg/kg to about 2 mg/kg.

82. The therapeutic product of claim 79, wherein the anti A β antibody concentration is about 10 mg/ml to about 60 mg/ml.

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83. The therapeutic product of claim 79, wherein the anti A β antibody concentration is about 20 mg/ml.

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84. The therapeutic product of claim 79, further comprising about 0.005% polysorbate 80.

85. The therapeutic product of claim 79, wherein the use is a subcutaneous administration.

25

86. The therapeutic product of claim 79, wherein the use is an intravenous administration.

FIG. 1

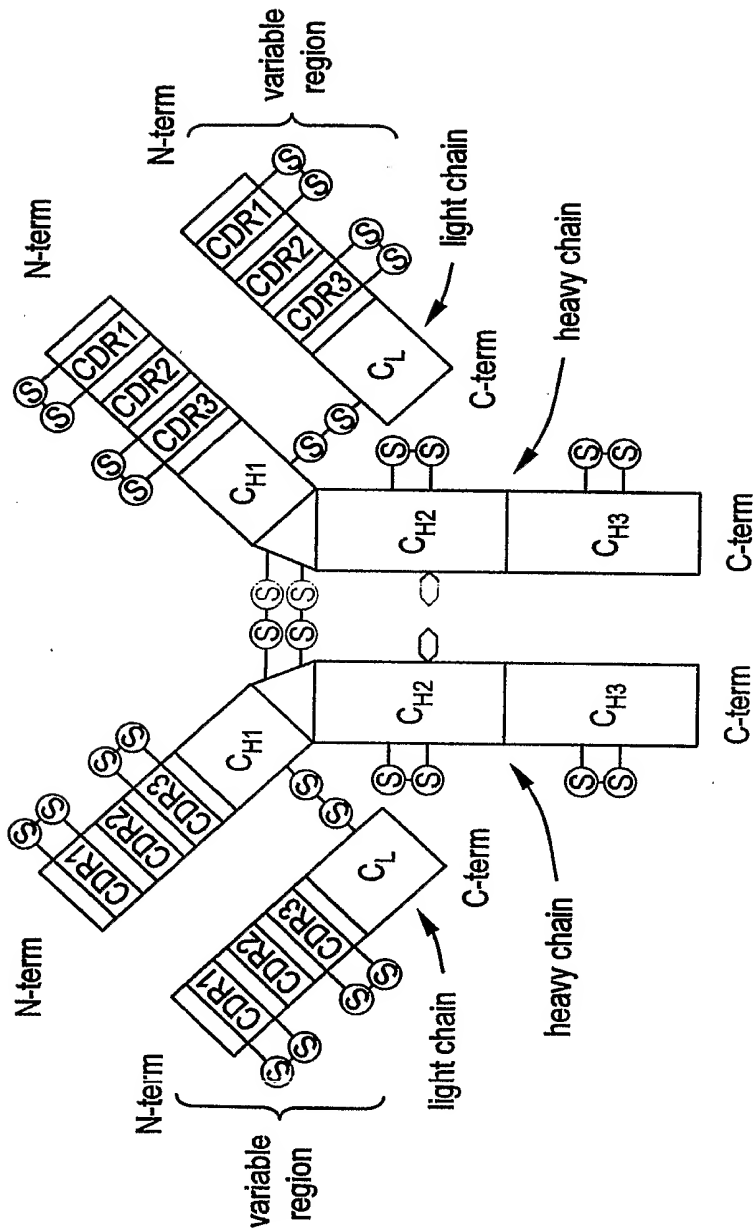


FIG. 2

Light Chain

1 DVVMTQSPLS LPVTPGEPAS ISCKSSQSLL DSDGKTYLNW LLQKPGQSPQ
 51 RLIYLVSKLD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
 101 RTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
 151 VQWKVDNALQ SGNSQESVTE QDSKDSITYSL SSTLTLSKAD YEKHKVYACE
 201 VTHQGLSSPV TKSFNRGEC

→ **Heavy Chain**

Heavy Chain

1 EVQLLESGG LVQPGSLRL SCAASGFTFS NYGMSWVRQA PGKGLEWVAS
 51 IRSGGGRTYY SDNVKGRFTI SRDNSKNTLY LQMSLRAED TAVYYCVRYD
 101 HYSGSSDYWG QGTLVTVSSA STKGPSVFFL APSSKSTSGG TAALGCLVKD
 151 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSVVTV PSSSLGTQTY
 201 ICNVNHHKPSN TKVDKKVEPK SCDKTHTCIP CPAPELLGGP SVFLFPPKPK

← **Light Chain** → **Heavy Chain**

251 DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 301 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 351 YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 401 DSDGSFFLYS KLTVDKSRWQ QGNVFSCVM HEALHNHYTQ KSLSLSPG(K)

FIG. 3A

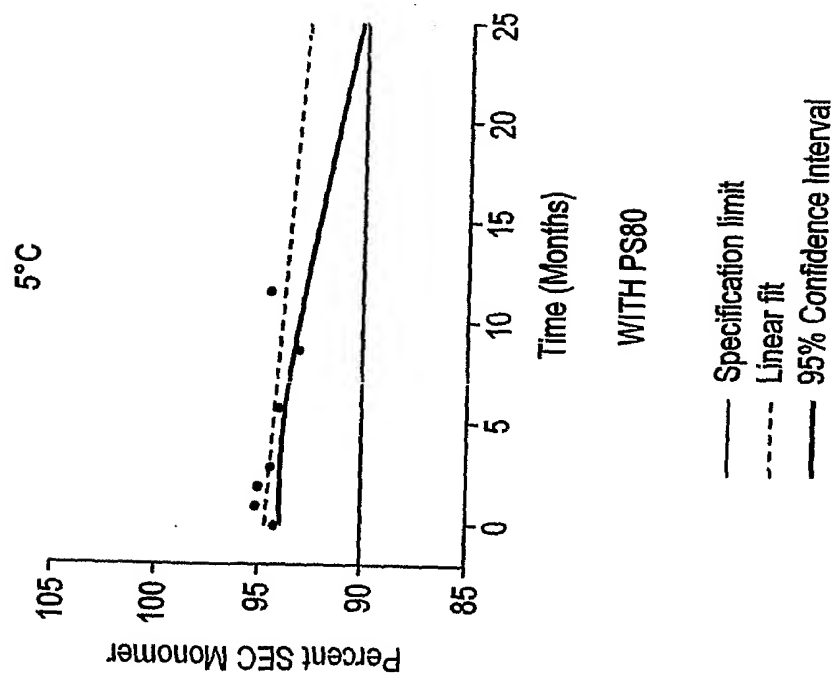


FIG. 3B

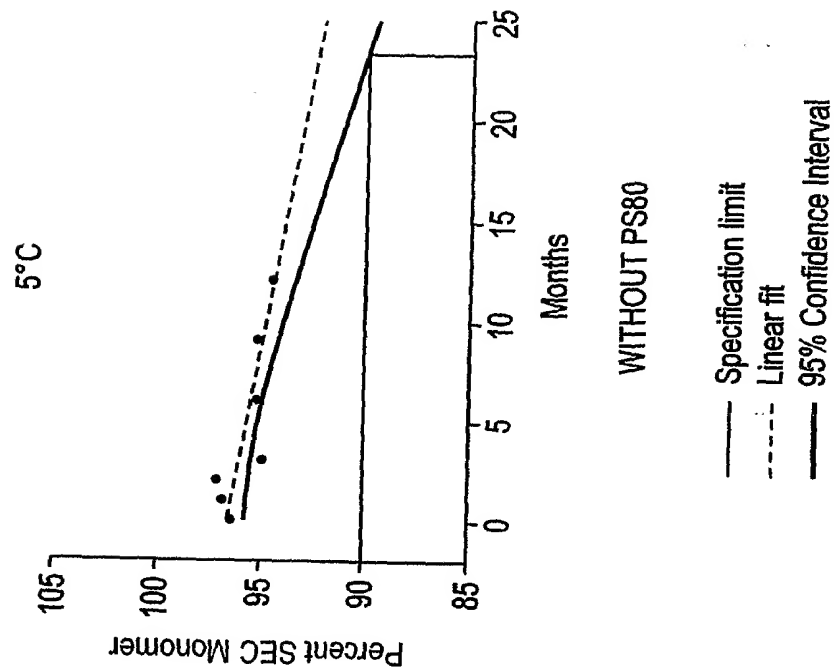


FIG. 4A

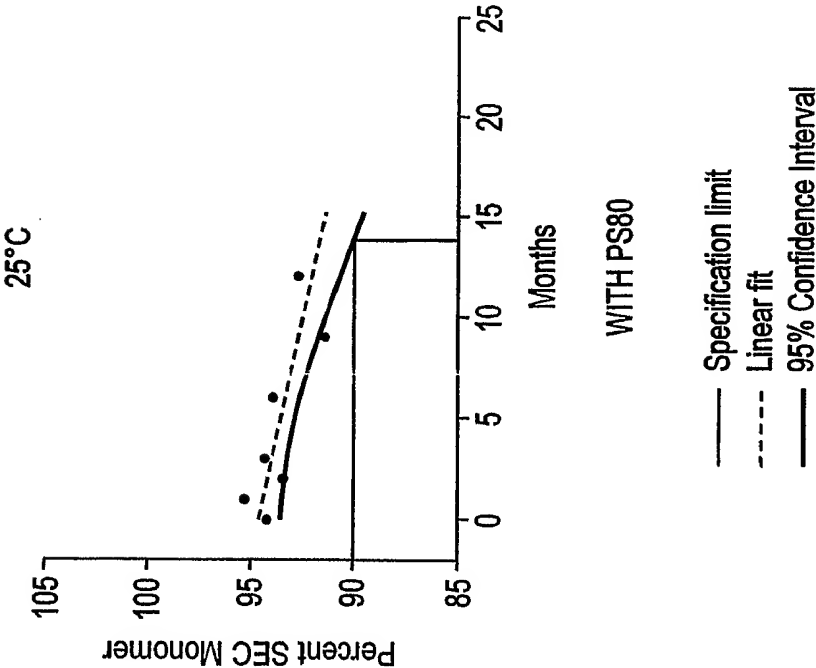


FIG. 4B

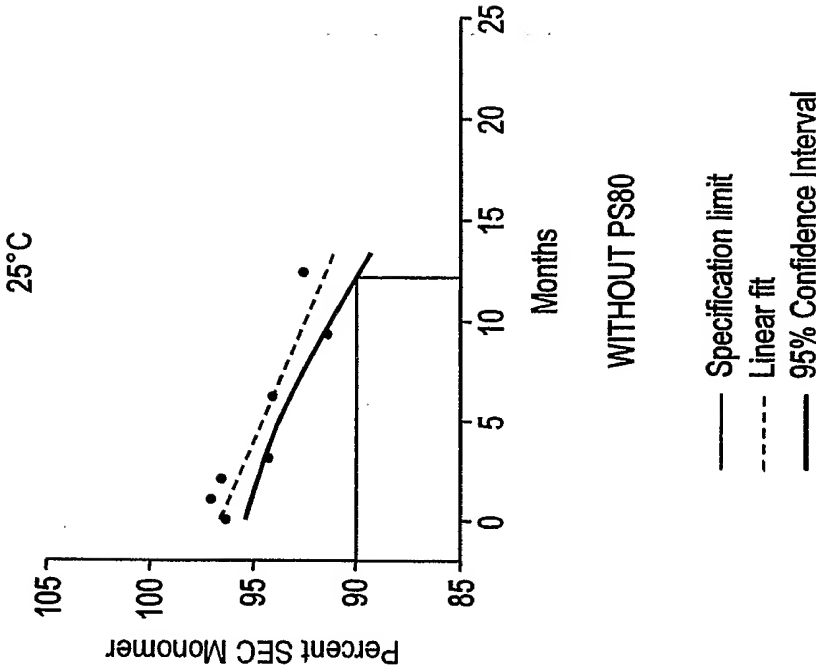


FIG. 5A

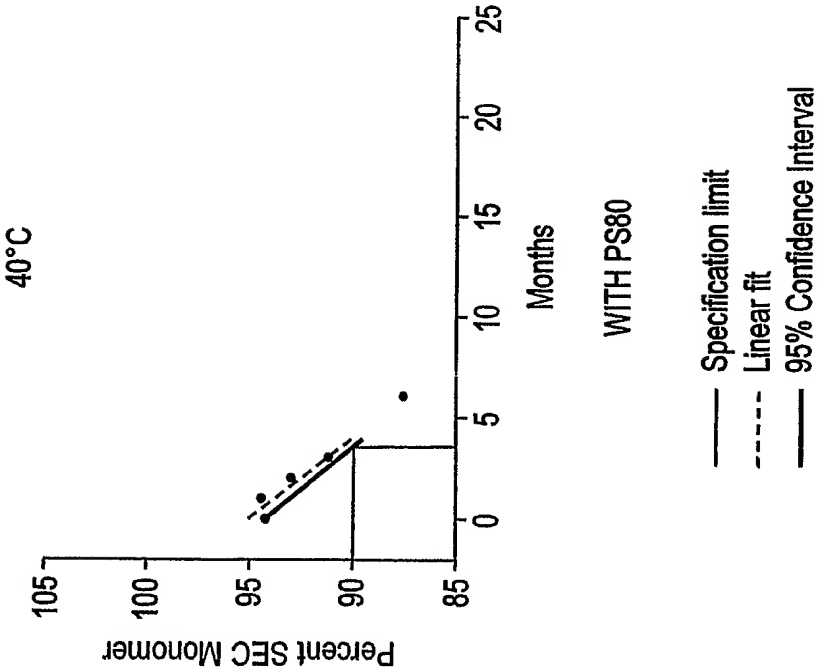


FIG. 5B

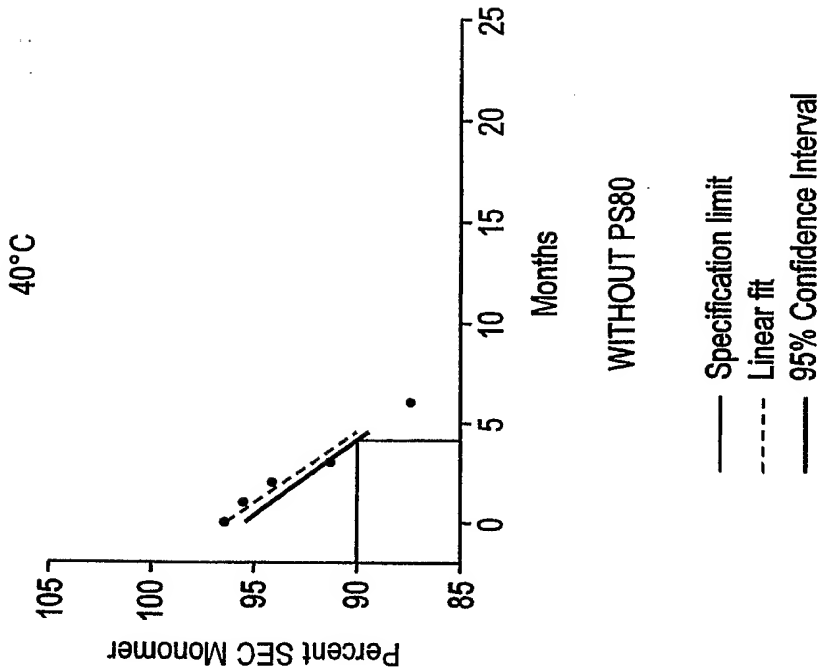


FIG. 6

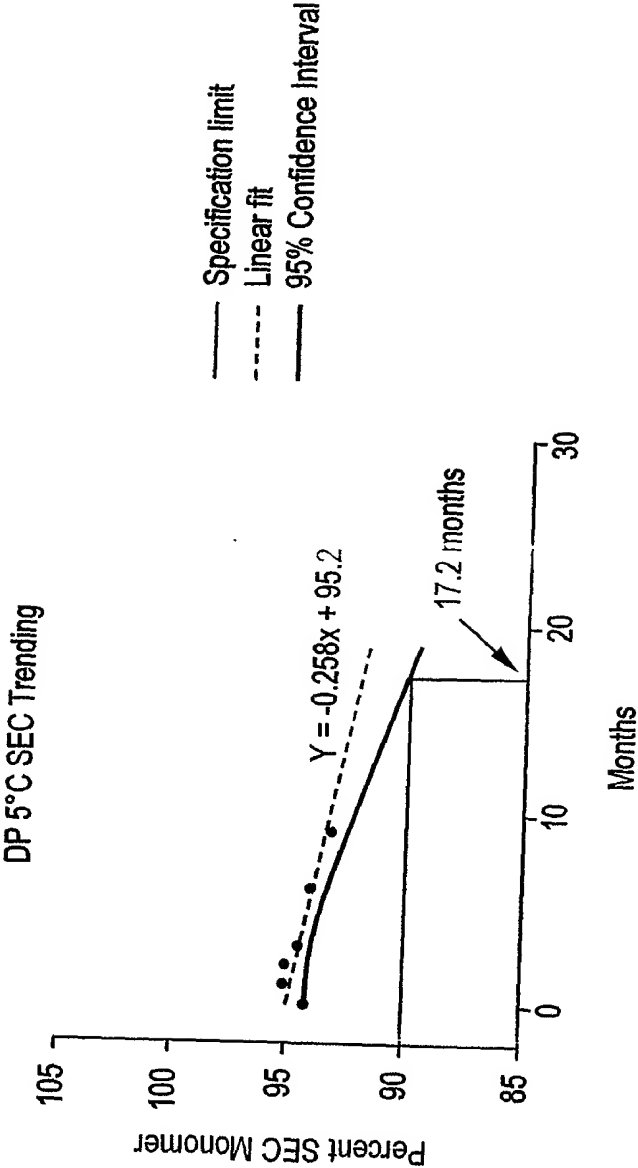


FIG. 7A

FIG. 7B

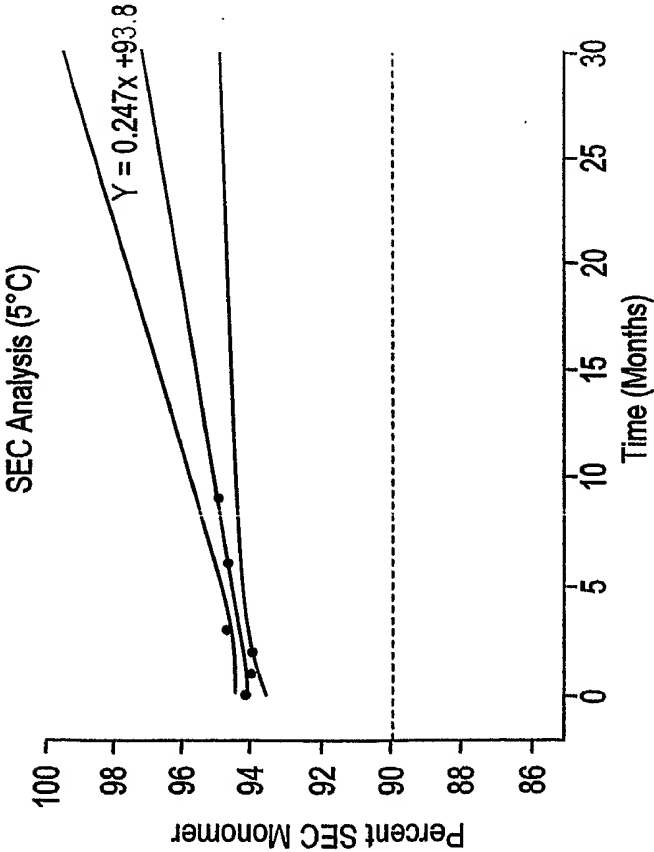
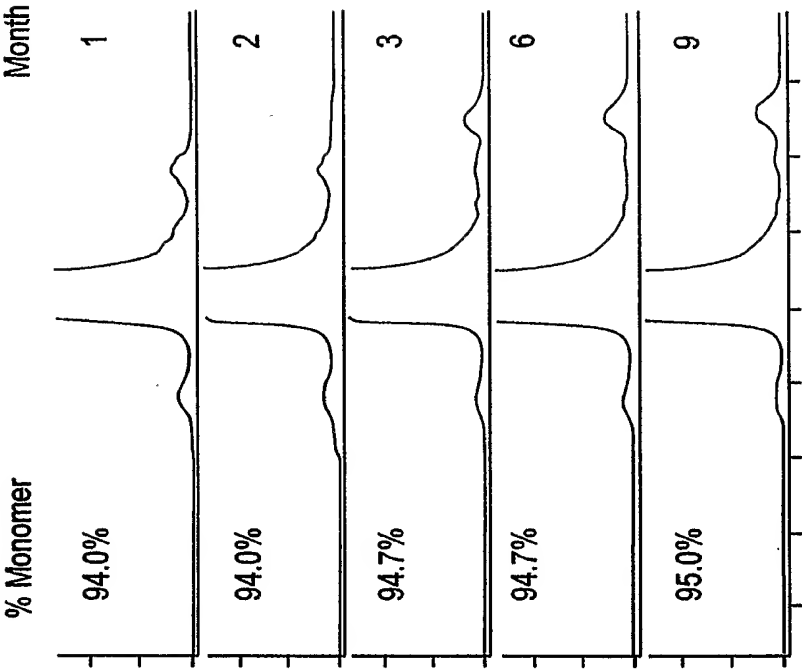


FIG. 8

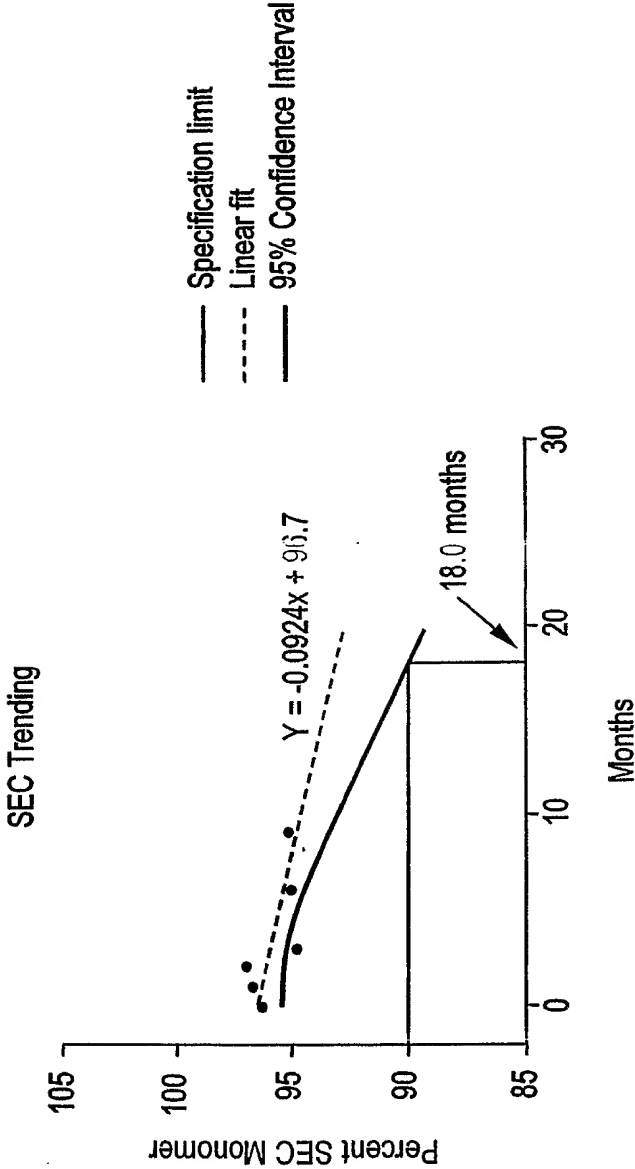


FIG. 9

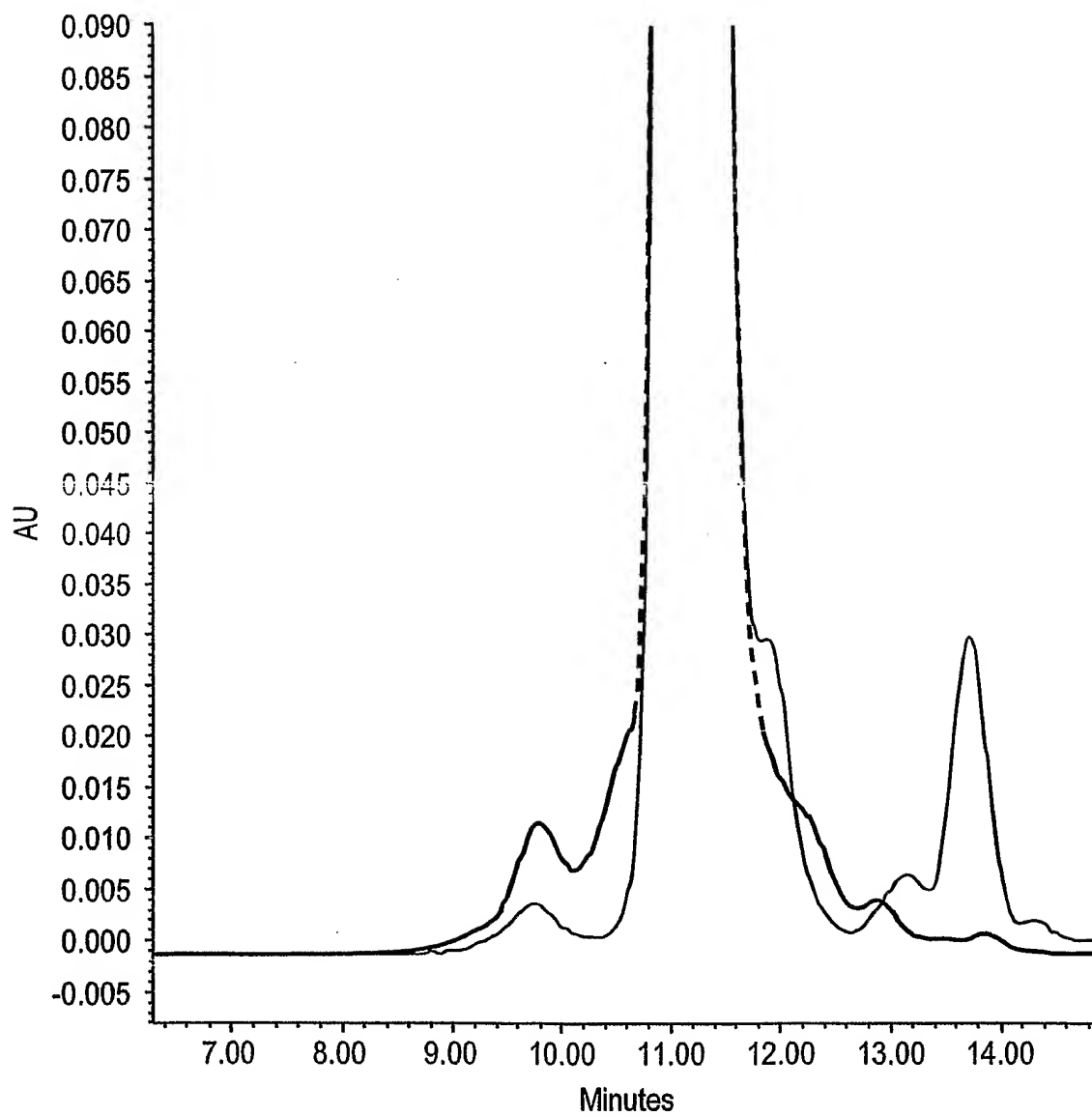
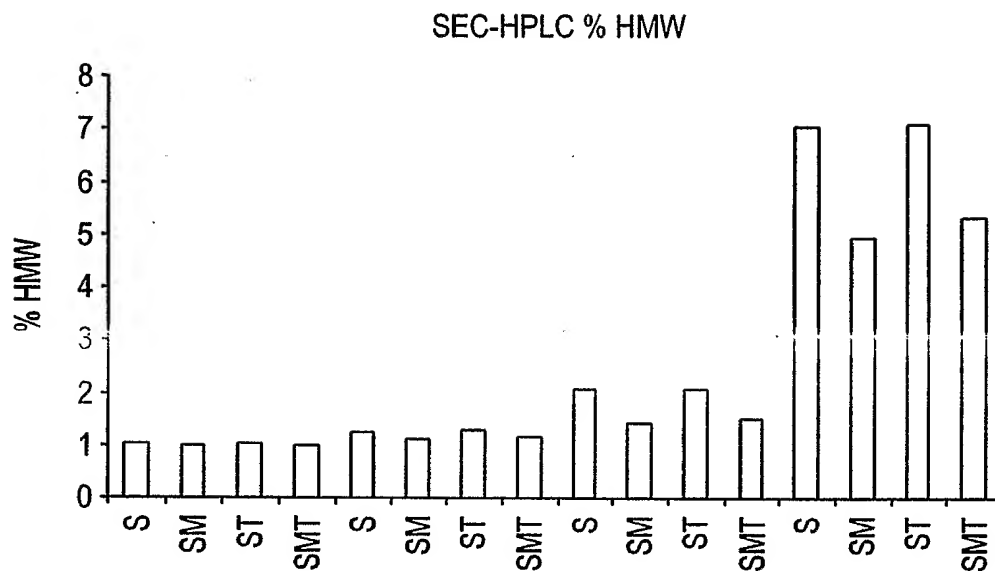
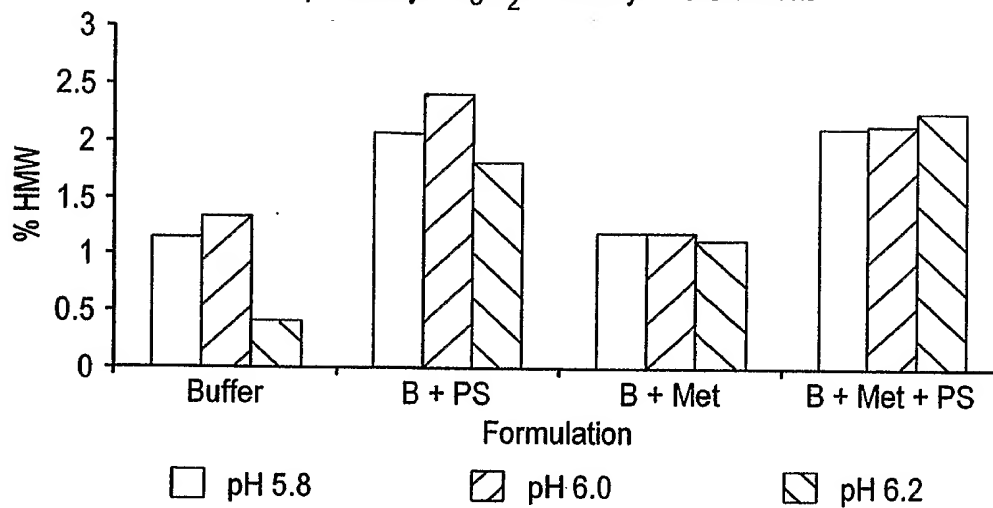
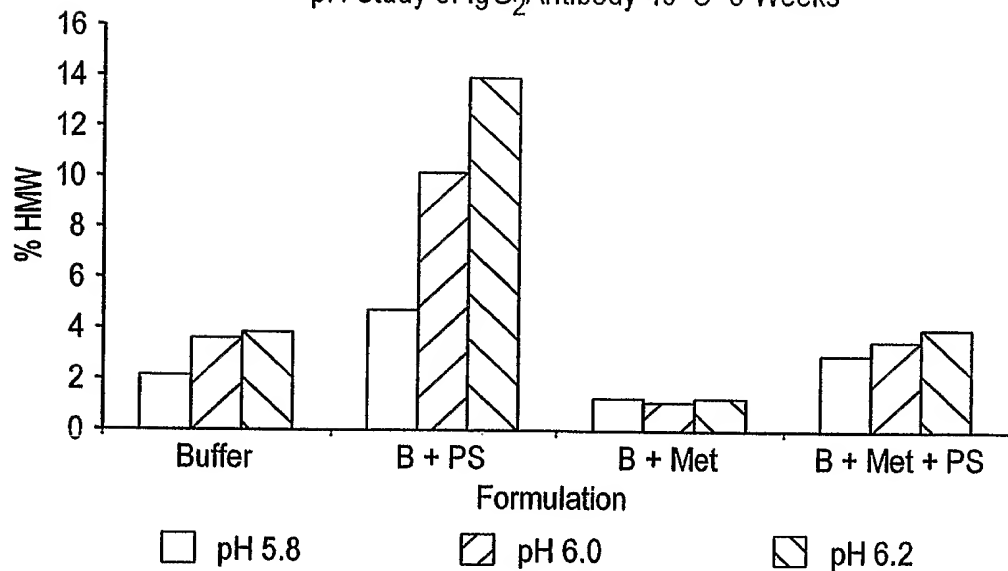


FIG. 10



S: 20 mM succinate pH 6.0
SM: succinate + 10 mM methionine
ST: succinate + 0.01% PS80
SMT: succinate + met + PS80
Concentration 14 mg/mL

FIG. 11ApH Study of IgG₂ Antibody 5°C 6 Weeks**FIG. 11B**pH Study of IgG₂ Antibody 40°C 6 Weeks

Buffer: 10 mM histidine, 150 mM NaCl
B+PS: 10 mM histidine, 150 mM NaCl, 0.01% PS80
B+Met: 10 mM histidine, 150 mM NaCl, 10 mM methionine
B+Met+PS: 10 mM histidine, 150 mM NaCl, 10 mM methionine, 0.01% PS80
Concentration 1 mg/mL

SEQUENCE LISTING

<110> LUISI, Donna
WARNE, Nicholas W.
KANTOR, Angela

<120> ANTI A BETA ANTIBODY FORMULATION

<130> ELN-046

<150> 60/648631

<151> 2005-01-28

<160> 2

<170> FastSEQ for Windows Version 4.0

 $\langle 210 \rangle$ 1

<211> 219

<212> PRT

<213> Artificial Sequence

 $\langle 220 \rangle$

<223> Synthetic Construct

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Asp 1	Val	Val	Met	Thr 5	Gln	Ser	Pro	Leu	Ser 10	Leu	Pro	Val	Thr	Pro 15	Gly
Glu	Pro	Ala	Ser 20	Ile	Ser	Cys	Lys	Ser 25	Ser	Gln	Ser	Leu	Leu 30	Asp	Ser
Asp	Gly	Lys 35	Thr	Tyr	Leu	Asn 40	Trp	Leu	Leu	Gln	Lys 45	Pro	Gly	Gln	Ser
Pro	Gln 50	Arg	Leu	Ile	Tyr 55	Leu	Val	Ser	Lys	Leu	Asp 60	Ser	Gly	Val	Pro
Asp 65	Arg	Phe	Ser	Gly 70	Ser	Gly	Ser	Gly	Thr 75	Asp	Phe	Thr	Leu	Lys 80	Ile
Ser	Arg	Val	Glu 85	Ala	Glu	Asp	Val	Gly	Val 90	Tyr	Tyr	Cys	Trp	Gln 95	Gly
Thr	His	Phe	Pro 100	Arg	Thr	Phe	Gly	Gln 105	Gly	Thr	Lys	Val	Glu 110	Ile	Lys
Arg	Thr	Val 115	Ala	Ala	Pro	Ser	Val 120	Phe	Ile	Phe	Pro	Pro 125	Ser	Asp	Glu
Gln	Leu 130	Lys	Ser	Gly	Thr	Ala 135	Ser	Val	Val	Cys	Leu 140	Leu	Asn	Asn	Phe
Tyr 145	Pro	Arg	Glu	Ala 150	Lys	Val	Gln	Trp	Lys 155	Val	Asp	Asn	Ala	Leu 160	Gln
Ser	Gly	Asn	Ser 165	Gln	Glu	Ser	Val	Thr 170	Glu	Gln	Asp	Ser	Lys 175	Asp	Ser
Thr	Tyr	Ser 180	Leu	Ser	Ser	Thr	Leu 185	Thr	Leu	Ser	Lys	Ala 190	Asp	Tyr	Glu
Lys	His 195	Lys	Val	Tyr	Ala	Cys	Glu 200	Val	Thr	His	Gln	Gly 205	Leu	Ser	Ser
Pro	Val 210	Thr	Lys	Ser	Phe 215	Asn	Arg	Gly	Glu	Cys					

 $\langle 210 \rangle$ 2

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 2

Glu	Val	Gln	Leu	Leu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25					30		
Gly	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ala	Ser	Ile	Arg	Ser	Gly	Gly	Gly	Arg	Thr	Tyr	Tyr	Ser	Asp	Asn	Val
	50					55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Val	Arg	Tyr	Asp	His	Tyr	Ser	Gly	Ser	Ser	Asp	Tyr	Trp	Gly	Gln	Gly
			100					105					110		
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
		115					120						125		
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
	130					135					140				
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
145					150					155					160
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
			165					170						175	
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180					185					190		
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
	210					215					220				
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
225					230					235					240
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
			245						250					255	
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
			260					265						270	
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
		275					280					285			
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
	290					295					300				
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
305					310					315					320
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
			325						330					335	
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
			340					345					350		
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
		355					360					365			
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
	370					375					380				
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
385					390					395					400
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
			405						410					415	
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
			420					425					430		
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
		435					440					445			

```

<210> SEQ ID NO 3
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: humanized antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa=Asp or Tyr
<220> FEATURE:
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<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa=Ser or Thr

<220> FEATURE:
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<223> OTHER INFORMATION: Xaa=Ser or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa=Leu, Ile, or Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: Xaa=Arg or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (88)..(88)
<223> OTHER INFORMATION: Xaa=Val or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (109)..(109)
<223> OTHER INFORMATION: Xaa=val or Leu

<400> SEQUENCE: 3

Xaa Val Val Met Thr Gln Xaa Pro Leu Xaa Leu Pro Val Thr Xaa Gly
1          5          10         15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20          25          30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
35          40          45

Pro Xaa Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50          55          60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80

Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Trp Gln Gly
85          90          95

Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys Xaa Glu Ile Lys
100         105         110

Arg

```

```

<210> SEQ ID NO 4
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa=Gln, Lys, or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (78)..(78)
<223> OTHER INFORMATION: Xaa=Ser or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (87)..(87)
<223> OTHER INFORMATION: Xaa=Arg or Lys
<220> FEATURE:
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<223> OTHER INFORMATION: Xaa=Ala, Ser or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (114)..(114)
<223> OTHER INFORMATION: Xaa=Leu, Thr, Ile, or Val

<400> SEQUENCE: 4

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Glu Val Xaa Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Xaa Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Xaa Xaa Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Xaa Val Thr Val Ser Ser
 115

<210> SEQ ID NO 5
 <211> LENGTH: 113
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: humanized antibody

<400> SEQUENCE: 5

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
 1 5 10 15
 Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
 20 25 30
 Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
 35 40 45
 Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
 85 90 95
 Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg

<210> SEQ ID NO 6
 <211> LENGTH: 119
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: humanized antibody

<400> SEQUENCE: 6

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Ser
 115

```

<210> SEQ ID NO 7
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized antibody

<400> SEQUENCE: 7
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1          5          10          15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20          25          30
Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
35          40          45
Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85          90          95
Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100         105         110
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115         120         125
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130         135         140
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145         150         155         160
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165         170         175
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180         185         190
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195         200         205
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210         215

```

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<210> SEQ ID NO 8
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized antibody

<400> SEQUENCE: 8

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Asn Tyr
20          25          30
Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser Asp Asn Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
100         105         110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115         120         125

```

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Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255
Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp
260 265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

```

Lys

```

<210> SEQ ID NO 9
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: humanized antibody
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<223> OTHER INFORMATION: Xaa=Val or Leu
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<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa=Ser or Thr
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<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa=Thr or Ser
<220> FEATURE:
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<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa=Gln, Asp, or Asn
<220> FEATURE:
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<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa=Ile or Val
<220> FEATURE:
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<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: Xaa=Arg or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (88)..(88)
<223> OTHER INFORMATION: Xaa=Val or Leu
<220> FEATURE:

```

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (105)..(105)
<223> OTHER INFORMATION: Xaa=Gly or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (109)..(109)
<223> OTHER INFORMATION: Xaa=Val or Leu

<400> SEQUENCE: 9

Asp Val Xaa Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Leu Gly
1      5      10      15
Xaa Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Xaa His Ser
20      25      30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35      40      45
Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50      55      60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65      70      75      80
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Phe Gln Gly
85      90      95
Ser His Val Pro Leu Thr Phe Gly Xaa Gly Thr Lys Xaa Glu Ile Lys
100     105     110

Arg

```

```

<210> SEQ ID NO 10
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: humanized antibody
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<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa=Gln or Glu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa=Val or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (64)..(64)
<223> OTHER INFORMATION: Xaa=Ser or Thr
<220> FEATURE:
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<222> LOCATION: (77)..(77)
<223> OTHER INFORMATION: Xaa=Lys or Arg
<220> FEATURE:
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<223> OTHER INFORMATION: Xaa=Ser or Thr
<220> FEATURE:
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<222> LOCATION: (83)..(83)
<223> OTHER INFORMATION: Xaa=Thr or Ser
<220> FEATURE:
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<222> LOCATION: (84)..(84)
<223> OTHER INFORMATION: Xaa=Met, Ile or Leu
<220> FEATURE:
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<223> OTHER INFORMATION: Xaa=Asn, Ser, Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Xaa=Met, Val, or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (118)..(118)
<223> OTHER INFORMATION: Xaa=Leu or Ser

<400> SEQUENCE: 10

```

```

Xaa Xaa Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Xaa
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Xaa Xaa Gln Val
65 70 75 80
Val Leu Xaa Xaa Thr Xaa Xaa Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
100 105 110
Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
115 120

```

<210> SEQ ID NO 11
 <211> LENGTH: 113
 <212> TYPE: PRT
 <213> ORGANISM: humanized antibody

<400> SEQUENCE: 11

```

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
20 25 30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95
Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg

```

<210> SEQ ID NO 12
 <211> LENGTH: 123
 <212> TYPE: PRT
 <213> ORGANISM: humanized antibody

<400> SEQUENCE: 12

```

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

```

<210> SEQ ID NO 13

<211> LENGTH: 219

<212> TYPE: PRT

<213> ORGANISM: humanized antibody

<400> SEQUENCE: 13

```

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1          5          10          15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Ile His Ser
20          25          30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35          40          45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50          55          60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85          90          95

```

Ser His Val Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> SEQ ID NO: 14
 <211> LENGTH: 453
 <212> TYPE: PRT
 <213> ORGANISM: humanized antibody

<400> SEQUENCE: 14

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala His Ile Tyr Trp Asp Asp Lys Arg Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
 65 70 75 80
 Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala Met Asp Tyr
 100 105 110
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125
 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 130 135 140
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 145 150 155 160
 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 165 170 175
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190
 Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 195 200 205
 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
 210 215 220
 Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 225 230 235 240
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 245 250 255
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 260 265 270
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 275 280 285
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Gln Gln Tyr Asn Ser
 290 295 300
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 305 310 315 320

```

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
    325                                330                                335
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
    340                                345                                350
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
    355                                360                                365
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                                375                                380
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    385                                390                                395                                400
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
    405                                410                                415
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    420                                425                                430
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    435                                440                                445
Leu Ser Pro Gly Lys
    450

```


<210> 15
 <211> 132
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: humanized 12B4VLv1

<220>
 <221> SIGNAL
 <222> (1)...(20)

<400> 6
 Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Ser
 -20 -15 -10 -5
 Gly Ser Ser Gly Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 1 5 10
 Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn
 15 20 25
 Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 30 35 40
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
 45 50 55 60
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
 65 70 75
 Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90
 Cys Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Gln Gly Thr Lys
 95 100 105
 Leu Glu Ile Lys
 110

<210> 16
 <211> 142
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: humanized 12B4VHv1

<220>
 <221> SIGNAL
 <222> (1)...(19)

<400> 8
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 -15 -10 -5
 Val Leu Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys
 1 5 10
 Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
 15 20 25
 Ser Thr Asn Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
 30 35 40 45
 Gly Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Glu Asp Lys Arg Tyr
 50 55 60
 Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys
 65 70 75
 Asn Gln Val Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
 80 85 90

Val Tyr Tyr Cys Ala Arg Arg Arg Ile Ile Tyr Asp Val Glu Asp Tyr
 95 100 105
 Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 110 115 120

<210> 17
 <211> 131
 <212> PRT
 <213> Mus musculus

<220>
 <221> SIGNAL
 <222> (1)...(19)

<400> 2
 Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
 -15 -10 -5
 Ser Ser Ser Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val
 1 5 10
 Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile
 15 20 25
 Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
 30 35 40 45
 Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
 50 55 60
 Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 65 70 75
 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys
 80 85 90
 Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
 95 100 105
 Glu Leu Lys
 110

<210> 18
 <211> 142
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: humanized 12B4VLv2

<220>
 <221> SIGNAL
 <222> (1)...(19)

<400> 10
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 -15 -10 -5
 Val Leu Ser Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys
 1 5 10
 Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
 15 20 25
 Ser Thr Asn Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
 30 35 40 45
 Gly Leu Glu Trp Ile Gly His Ile Tyr Trp Asp Glu Asp Lys Arg Tyr
 50 55 60
 Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Lys Asp Thr Ser Lys
 65 70 75

```

      Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
      80      85      90
Val Tyr Tyr Cys Ala Arg Arg Arg Ile Ile Tyr Asp Val Glu Asp Tyr
  95      100      105
Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
110      115      120

```

<210> 19
 <211> 142
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: humanized 12B4VLv3

<220>
 <221> SIGNAL
 <222> (1)...(19)

```

<400> 12
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
      -15      -10      -5
Val Leu Ser Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys
      1      5      10
Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu
  15      20      25
Ser Thr Asn Gly Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys
30      35      40      45
Gly Leu Glu Trp Leu Gly His Ile Tyr Trp Asp Glu Asp Lys Arg Tyr
      50      55      60
Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Lys Asp Thr Ser Lys
      65      70      75
Asn Gln Val Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
      80      85      90
Val Tyr Tyr Cys Ala Arg Arg Arg Ile Ile Tyr Asp Val Glu Asp Tyr
  95      100      105
Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
110      115      120

```

<210> 20
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v1 - VL region

```

<400> 7
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
  1      5      10      15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
      20      25      30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
      35      40      45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
  50      55      60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65      70      75      80

```

```

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Ser
      85          90          95
Ser His Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
      100          105          110

```

<210> 21
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> h12A11v1 - VH region

```

<400> 10
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 22
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v2

```

<400> 13
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 23
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v2.1

<400> 14
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 24
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v3

<400> 15
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 25
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic construct, humanized 12A11 v3.1 VH

<400> 36

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
          20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
          35          40          45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
          50          55          60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Leu
65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
          85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
          100          105          110
Gly Thr Thr Val Thr Val Ser Ser
          115          120

```

<210> 26

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v4.1

<400> 16

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
          20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
          35          40          45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
          50          55          60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
          85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
          100          105          110
Gly Thr Thr Val Thr Val Ser Ser
          115          120

```

<210> 27

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v4.2

<400> 17

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
          20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

```

```

      35              40      45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
  50              55              60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
  65              70              75
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85              90              95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100              105              110
Gly Thr Thr Val Thr Val Ser Ser
      115              120

```

<210> 28

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v4.3

<400> 18

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1              5              10              15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20              25              30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35              40              45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50              55              60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
      65              70              75
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85              90              95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100              105              110
Gly Thr Thr Val Thr Val Ser Ser
      115              120

```

<210> 29

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v4.4

<400> 19

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1              5              10              15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20              25              30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35              40              45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50              55              60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
      65              70              75
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85              90              95

```

```

      100      105      110
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
Gly Thr Thr Val Thr Val Ser Ser
      115      120

```

<210> 30

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v5.1

<400> 20

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
      65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 31

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v5.2

<400> 21

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
      65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 32

<211> 121
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v5.3

<400> 22
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser Val
 115 120

<210> 33
 <211> 121
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v5.4

<400> 23
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser Val
 115 120

<210> 34
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v5.5

<400> 24

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
      65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 35

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v5.6

<400> 25

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20          25          30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35          40          45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50          55          60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
      65          70          75          80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85          90          95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100          105          110
Gly Thr Thr Val Thr Val Ser Ser
      115          120

```

<210> 36

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A116.1

<400> 26

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
      20          25          30

```



```

      85                      90                      95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100                      105                      110
Gly Thr Thr Val Thr Val Ser Ser
      115                      120

```

<210> 39
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v6.4

```

<400> 29
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1                      5                      10                      15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
      20                      25                      30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35                      40                      45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50                      55                      60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
      65                      70                      75                      80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85                      90                      95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100                      105                      110
Gly Thr Thr Val Thr Val Ser Ser
      115                      120

```

<210> 40
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v7

```

<400> 30
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1                      5                      10                      15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
      20                      25                      30
Gly Met Ser Val Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
      35                      40                      45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
      50                      55                      60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
      65                      70                      75                      80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
      85                      90                      95
Cys Ala Arg Arg Thr Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
      100                      105                      110
Gly Thr Thr Val Thr Val Ser Ser
      115                      120

```

" h_{max} h_{min} h_{max} h_{min}
 <210> 41

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v8

<400> 31

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Phe	Ser	Gly	Phe	Ser	Leu	Ser	Thr	Ser
			20					25					30		
Gly	Met	Ser	Val	Gly	Trp	Ile	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu
		35					40					45			
Trp	Leu	Ala	His	Ile	Trp	Trp	Asp	Asp	Asp	Lys	Tyr	Tyr	Asn	Pro	Ser
	50					55					60				
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Asn	Ser	Lys	Asn	Thr	Val
65					70					75				80	
Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr
			85						90					95	
Cys	Ala	Arg	Arg	Thr	Thr	Thr	Ala	Asp	Tyr	Phe	Ala	Tyr	Trp	Gly	Gln
			100					105						110	
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
			115				120								

```

<210> SEQ ID NO 42
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized antibodies
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa at position 2 is Val or Ile
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa at position 7 is Ser or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa at position 14 is Thr or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa at position 15 is Leu or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa at position 30 is Ile or Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: Xaa at position 50 is Arg, Gln, or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (88)..(88)
<223> OTHER INFORMATION: Xaa at position 88 is Val or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (105)..(105)
<223> OTHER INFORMATION: Xaa at position 105 is Gln or Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (108)..(108)
<223> OTHER INFORMATION: Xaa at position 108 is Lys or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (109)..(109)
<223> OTHER INFORMATION: Xaa at position 109 is Val or Leu
<400> SEQUENCE: 42
Asp Xaa Val Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Xaa Gly
1          5          10          15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Xaa Tyr Ser
20          25          30
Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35          40          45
Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Ser Gln Ser
85          90          95
Thr His Val Pro Trp Thr Phe Gly Xaa Gly Thr Xaa Xaa Glu Ile Lys
100         105         110
Arg

```

```

<210> SEQ ID NO 43
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized antibodies
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa at position 1 is Glu or Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa at position 7 is Ser or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (46)..(46)
<223> OTHER INFORMATION: Xaa at position 46 is Glu, Val, Asp or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (63)..(63)
<223> OTHER INFORMATION: Xaa at position 63 is Thr or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (75)..(75)
<223> OTHER INFORMATION: Xaa at position 75 is Ala, Ser, Val or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (76)..(76)
<223> OTHER INFORMATION: Xaa at position 76 is Lys or Arg

```

```

<220> FEATURE: "MISC_FEATURE"
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (89)..(89)
<223> OTHER INFORMATION: Xaa at position 89 is Glu or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (107)..(107)
<223> OTHER INFORMATION: Xaa at position 107 is Leu or Thr

```

<400> SEQUENCE: 43

```

Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20          25          30
Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
35          40          45
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
100         105         110

```

```

<210> SEQ ID NO 44
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized antibodies

```

<400> SEQUENCE: 44

```

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1          5          10          15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile Tyr Ser
20          25          30
Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35          40          45
Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
85          90          95
Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100         105         110

```

Arg

```

<210> SEQ ID NO 45
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized antibodies

```

<400> SEQUENCE: 45

```

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20          25          30
Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35          40          45
Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65          70          75          80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100         105         110

```

<210> SEQ ID NO 46
 <211> LENGTH: 219
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence

<220> FEATURE:
 <223> OTHER INFORMATION: Humanized antibodies

<400> SEQUENCE: 46

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
 1 5 10 15
 Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile Tyr Ser
 20 25 30
 Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
 85 90 95
 Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> SEQ ID NO 47
 <211> LENGTH: 442
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Humanized antibodies

<400> SEQUENCE: 47

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
 20 25 30
 Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
 35 40 45
 Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 100 105 110
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 115 120 125
 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 130 135 140
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 145 150 155 160
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 165 170 175
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 180 185 190

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 195 200 205
 Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 210 215 220
 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 225 230 235 240
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 245 250 255
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 260 265 270
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 275 280 285
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 290 295 300
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 305 310 315 320
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 325 330 335
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 340 345 350
 Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 355 360 365
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 370 375 380
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 385 390 395 400
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 405 410 415
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 420 425 430
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440